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Outbreak investigation and molecular characterization of African horse sickness virus circulating in selected areas of Ethiopia

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ABSTRACT

The study was conducted from June 2011 to May 2012 in central, northern and western parts of Ethiopia to investigate and identify circulating serotypes of African horse sickness virus (AHSV). The indigenous knowledge of equine owners about AHS in the study areas was assessed and also the retrospective data of AHS outbreaks for 2011 were analyzed. Whole blood samples were collected for virus isolation and serotyping from diseased horses and mules showing typical signs of the AHS. Virus isolation on Vero cell and detection of AHSV genomes using conventional RT-PCR were conducted. Further molecular characterization and serotyping were done on positive isolates. The questionnaire survey revealed that equine owners do recognize AHS clinically and have a local name that varies in different regions. From the 72 equine owners interviewed about their knowhow of AHS, 48 (66.7%) of respondents were not aware of AHS disease mode of transmission. The retrospective disease report data showed that a total of 208 outbreaks were reported and 3036 cases and 1167 deaths were recorded in 2011. AHS outbreaks were more frequently observed from September to December and the highest number of outbreaks was recorded in October. During the study period totally six outbreaks were investigated and a total of 62 horses and 10 mules were found sick and all the four forms of AHS were observed. Cardiac form accounted for 52.8%, followed by African horse sickness fever form 31.9%, pulmonary form 8.4% and mixed form 6.9%. AHSV-9 was the only serotype circulating in the outbreak areas.

1. Introduction

Agriculture is the dominant economic feature of Ethiopia of which livestock is an essential component. In the livestock sector equines play an important role in the economy of the nation. The most important feature of animal transport in Ethiopia is the use of donkeys, horses and mules as pack animals, for pulling carts and for riding. They transport hugely diverse items including people, agricultural products, food, water and construction materials. They have multiple uses, which are not only limited to economic aspects, but also attributed to socio-cultural values. These beasts of burden dramatically reduced the domestic transport burden of rural people, especially women, and have created employment and income-generation opportunities for many people. Studies have shown that transport constitutes one of the necessary inputs for rural development and has a positive stimulus for growth in food production, poverty alleviation and overall communication (Pearson, 2000; Pearson et al., 1999).

As per results from the livestock survey in Ethiopia, there are about 2.03 million horses, 6.21 million donkeys and 0.38 million mules (CSA, 2012). Despite their importance, equids are the most neglected and affected animals by a series of health and welfare problems (Shelima et al., 2007). Among infectious diseases which cause severe socio-economic losses to the equine owning population and the national economy in general are African horse sickness (AHS) and epizootic lymphangitis (Admasu and Shiferaw, 2011).

African Horse Sickness (AHS) (*Peste equina africana*, *Peste equine*) is an insect-borne, viral disease characterized by severe pyrexia, wide spread hemorrhage and edematous exudations (OIE, 2012). AHS virus affects all species of *Equidae* family (Horses, Mules, Donkeys and Zebras). Horses are the most susceptible to AHS with a

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mortality rate of 50–95% followed by mules with mortality around 50%. In enzootic regions of Africa, donkeys are very resistant to AHS and experience only subclinical infections (Guthrie, 2008; OIE, 2012).

AHS is caused by African horse sickness virus (AHSV) which is a member of the genus *Orbivirus* in the family *Reoviridae* (Radostits et al., 2007). AHSV has nine distinct strains, all morphologically similar to other orbiviruses such as bluetongue virus, epizootic haemorrhagic disease virus and equine encephalosis virus (EEV) which have similar morphological and biochemical properties with distinctive pathological and antigenic properties as well as host ranges (Roy and Sutton, 1998). AHSV has a genome consisting of 10 segments of double-stranded RNA (Boinas et al., 2009). These segments code for the seven structural proteins (VP1 to 7) and four non-structural proteins (NS1, NS2, NS3 and NS3A). Most of the genome segments coded for only one protein (Grubman and Lewis, 1992). AHSV is transmitted by the bite of blood-sucking insects including midges (*Culicoides* spp.), mosquitoes and large biting flies in the genera *Stomoxys* and *Tabanus* (Mellor, 1993, 1994; Wilson et al., 2009), while, *Culicoides imicola* is the principal vector responsible for the transmission of AHSV within its enzootic area and possibly during epizootics (Cetre-Sossah et al., 2004; Venter et al., 2000; Wilson et al., 2009).

AHS can be diagnosed based on clinical signs and characteristic lesions combined with an appropriate history and epidemiological information. However, other signs and lesions are less specific for sub-acute form of AHS, and other diseases such as equine encephalosis, equine infectious anaemia, equine morbillivirus pneumonia, equine viral arteritis, babesiosis and purpura haemorrhagica may be confused with one or other forms of AHS and should be excluded (Mellor and Hamblin, 2004; OIE, 2012). At laboratory level African horse sickness can be diagnosed by isolating the virus or detecting its nucleic acids or antigens (Mellor and Hamblin, 2004; OIE, 2012). Virus isolation is particularly important when outbreaks are seen outside endemic areas. AHSV can be isolated in embryonated eggs, by intracerebral inoculation of newborn mice, or in cell cultures. Suitable cultures for inoculation include baby hamster kidney (BHK-21), monkey stable (MS) and African green monkey kidney (Vero) cells. AHSV antigens can be detected with ELISAs (Crafford et al., 2011, 2003). A reverse-transcription polymerase chain reaction (RT-PCR) technique is used to detect viral RNA. A recently developed type-specific RT-PCR assay can be used for rapid serotyping (Rodriguez-Sanchez et al., 2008; Sailleau et al., 2000, 1997).

The disease is confined to sub-Saharan Africa, although periodic epizootics have caused severe outbreaks of the disease outside enzootic regions, i.e., North Africa, the Middle East, and Southern Europe (Boinas et al., 2009; Coetzer and Guthrie, 2004; Fernando et al., 2009; Mellor, 1994; Mellor and Hamblin, 2004; OIE, 2012; Williams et al., 1998). An epidemiological survey conducted in Ethiopia between 1977 and 1981 indicated that majority of the previous outbreaks were due to serotype 9. To comply with, National Veterinary Institute (NVI), Debre Ziet, Ethiopia had also been producing attenuated monovalent freeze dried AHS vaccine against this dominant circulating serotype of the virus (AHSV-9) for years. However, outbreak reports were increasing despite increasing vaccination coverage in some of regions. This prompted the necessity of field outbreak investigation. In 2008 fifteen outbreaks occurred in Keficho Shekicho and Bench Maji zones of SNNPRS and Illubabor zone of Oromia and killed 2185 equids. Samples from these outbreaks were submitted to the OIE's reference laboratory, Onderstpoort Veterinary Institute, South Africa and AHSV serotype 2 was identified for the first time in the country (Pan African Animal Health Yearbook, 2008). Quite recently Aklilu et al. (2012) also reported the presence of five serotypes (9, 8, 6, 4, 2) in the country. Therefore the ever-increasing diversity of serotypes and wide occurrence of AHS outbreaks necessitated further investigation for possible isolation and identification of other serotypes of AHSV and also to understand the seasonality of the disease and identify and map areas which are highly affected by AHS disease in the country.

2. Materials and methods

2.1. Study area

Outbreaks of AHS were investigated in central, western, northern and southern parts of Ethiopia. In central Ethiopia, outbreaks in East Shewa (Ada'a districts) were investigated. Outbreaks in western Ethiopia in Jima zone of Oromia region and in southern Ethiopia, Welayta zone (Sodo district) of the Southern Nation Nationalities and People's Regional State (SNNPRS) and from northern Ethiopia, outbreaks in West Gojjam (Bahrdar and Mecha districts) and Awi zone (Dangla district) were investigated (Fig. 1).

2.1.1. Study design

The study design is a combination of retrospective, questionnaire survey and active disease search in response to outbreak reported by district animal health professionals.

2.1.2. Retrospective data analysis

The retrospective data of outbreaks of AHS reported to the Animal and Plant Health Regulatory Directorate (APHRD), Ministry of Agriculture (MOA) office from all over the country were obtained for the year 2011 and analyzed to see national representation of AHS outbreaks and months of the year when the disease more frequently occurred.

2.1.3. Questionnaire survey

A semis-structured questionnaire was used to gather information from respondents in the study areas. The format was employed so as to assess the indigenous knowledge of owners on AHS, risk factors such as management practices, presence of equine biting insects, degree of susceptibility of the three equine species, age, season and agro-ecology of the areas were gathered from the 72 respondents owning at least either a donkey, horse, or mule.

2.1.4. Active disease search

When an active outbreak of AHS was reported from the field veterinarians an investigation was conducted at the specific site and epidemiological information was gathered by interviewing equine owners and district animal health professionals. Clinical examination of cases and data were recorded.

2.1.5. Sample collection

Equine manifesting suspected clinical signs of African horse sickness were thoroughly examined and whole blood was collected from jugular vein using sterile EDTA vaccutainer tube. During clinical examinations, a total of 72 equines were found sick and whole blood sample was collected from 52 of visited animals. Identification number was given to each sample including date of collection, district, peasant association and species of animal. The samples were transported under cold chain to the National Veterinary Institute, Debre zeit and stored at +4 °C for virology and molecular analysis.

2.1.6. Laboratory diagnosis

2.1.6.1. Virus isolation. Virus isolation was conducted in virology diagnostic laboratory of the NVI. Virus isolation was conducted using the following protocol. Three ml blood was taken from each sample and mixed with equal volume of sterile PBS in a sterile