Prevalence of African Horse Sickness in Donkeys in Three Northern Regions of Cameroon

Mohamed M. F. Ndebé, Mohamed M. M. Mouiche, Mildred N. Tangu, Frédéric Moffo, Rodrigue Simonet Namegni Poueme, and Julius Awah-Ndukum

ABSTRACT

African Horse Sickness (AHS) is a non-contagious viral disease that is endemic in sub-Saharan Africa countries and poses an important production and health threats of equines. AHS can induce up to 95% mortality in horses and 10% in donkeys. However, there is dearth of information of its epidemiology in donkeys in Cameroon. This study was therefore carried out to determine the prevalence and associated risk factors of AHS in donkey in three Northern regions of Cameroon. Donkey sera were subjected to the ELISA blocking test for the determination of antibodies against African Horse Sickness Virus (AHSV) and positive samples were subjected to Capture-ELISA to determine the presence of antigens. Spleen samples were collected from fifteen donkey carcasses, of unknown causes of death, were screened using capture ELISA and positive reactors confirmed using conventional PCR. Potential risk factors associated with AHS were assessed based on the information collected in the field. The Chi-Square test and the Odd ratio were used to test the association between prevalence and the different variables. Of the 266 sera obtained, 219 were positive for anti-AHS virus antibodies corresponding to a sero-prevalence of 82.33% [95% CI: 77.30 – 86.44] and no antigen detected from the corresponding whole blood. Locality (p<0.001, χ² = 22.96) and age (p<0.001, χ² = 24.31) significantly influenced sero-positivity of the donkeys. The sero-prevalence was higher (p<0.05) in the North (87.18% [95% CI: 81.03 – 91.55]) and Far North regions (84.81% [95% CI: 75.30 – 91.09]) than in the Adamawa region (51.61% [95% CI: 34.84 – 68.03]). Donkeys that were at least 3 years old (89.42% [95% CI: 84.22 – 93.05]) were at higher risk of AHS (p<0.0001, χ² = 22.54, OR: 4.56 [95% CI: 2.36 – 8.82]) compared to young animals of less than 3 years old (32.14% [95% CI: 27.15-37.13, OR: 0.15]). The study highlights that AHS is highly endemic in all age groups and both sexes of donkey populations in Northern Cameroon and donkeys maybe play important roles in the maintenance of the disease in equine populations in the country.

Keywords: African Horse Sickness, Cameroon, donkeys, prevalence, risk factors.

I. INTRODUCTION

African horse sickness (AHS) is a non-contagious fatal arboviral disease of horses and other equids caused by an arbovirus of the family Reoviridae [1], [2]. AHS affects all equine species [3], solipeds [4] and carnivores accidentally [5], [6]. Though the main transmitting vectors are Culicoids, iatrogenical transmission assisted by mechanical vectors such as mosquitoes, Stomox and Tabanidae have been reported [7]-[10]. The pathogenesis, clinical pathology and symptoms of AHS in horse and other equines [2], [3], [9]-[13], climatic influences on the activities the transmitting vectors in relation to increasing the incident of AHS [14]-[16] have been widely described. The applications of preventive measures, environmental hygiene and anti-AHS vaccinations to control the incidence of the disease in infected communities have been reported [2], [10], [17]-[19]. The climate of many African countries provides for high temperatures, mild to high humid conditions, wet to muddy soils and vegetation cover that maintains hygrometric conditions favouring continuous presence of vectors throughout the year [14], [16].
Also, the use of live vaccines with the risk of reversion to virulent strains may contribute to AHS occurrence in non-enzootic areas [17]. The disease is endemic in sub-Saharan Africa countries where it poses an important production and health threats of equines, inducing up to 95% mortality in horses and 10% in donkeys [3].

AHS is endemic in sub-Saharan Africa and the estimated sero-prevalence of antibodies in horses of 86.6% [20], 81.0% [21], 46.6% [22] and 58.93% [23] was reported in Nigeria, Gambia, Ethiopia and Cameroon, respectively. Also, estimated anti-AHS antibodies prevalence in donkeys of 24.6% in Ethiopia [24], 73% in Uganda [25] and 72.6% in Burkina-faso [26] has been reported. However, there is dearth of information regarding the profile of AHS in donkey in Cameroon and a surveillance program is essential to provide knowledge on the epidemiology and factors contributing to the spread of AHS in domestic equine species in the country.

Therefore, this study was carried out to determine the sero-prevalence and associated risk factors of AHS in the Northern Regions of Cameroon.

II. MATERIALS AND METHODS

A. Study Area and Study Design

The present study is cross-sectional survey carried out from March to June 2019 to determine the sero-prevalence and associated risk factors of AHS in three Northern Regions of Cameroon. The three Northern regions include the Far-North (10° - 12° N and 14° - 15° E) and North (8° – 10° N and 12° - 14° E) regions of the Sudano-Sahelian agro-ecological and the Adamawa (5° - 8° N and 11° - 14° E) region of the high Guinean savannah agro-ecological zone of the country. These three regions contribute over 95.88% of the national donkey herd [27]. The Sudano-Sahelian zone has low altitudes with the highest peak being 1442 m, an average temperature of 28 °C (maxima of 40 °C to 45 °C) and a monomodal rainfall of 400 to 1200 mm per year. The high Guinean savannah zone is characterized by a vast plateau of altitudes between 900 and 1500 m, humid tropical type Sudanese climate and two seasons (dry and rain) per year. The average annual rainfall is about 1500 mm while the temperatures are moderate and range from 20 °C to 26 °C [28].

B. Sample Size and Data Collection

A sample size of 199 donkeys was estimated [29] based on previous reports on the prevalence of AHS in horse of 84.76% in Cameroon [23] with a confidence interval of 95% and precision set at 5%. The study used a stratified random technique. Sampling of each region was according to their proportion in the national herd size. Random number generation technique was used for the selection of herd from a list obtained at the Delegations of Livestock, Fisheries and Animal Industries in the study regions and completed by private field veterinary practitioners. The scientific research and ethics committee of the School of Veterinary Medicine and Sciences of the University of Ngaoundéré – Cameroon provided ethical approval for this research. Following explanation of the purpose of study to donkey owner in these regions, they provided verbal informed consents before they and their animals were included in the study. The information’s related to the donkeys were collected.

Apart from procedural restraining manipulations for safety purposes and jugular venepuncture for blood sampling (>15 mL) using sterile vacutainer, the animals were not subjected to suffering. Briefly, for each selected animal, blood samples were collected in test tubes without anti-coagulant and a test tubes containing Ethylene Diamine Tetra-Acetic Acid (EDTA) and then transported to the National Veterinary Laboratory, Bokle, Garoua Cameroon. Blood samples were centrifuged and sera obtained were used for detection of the antibodies using ELISA Blocking (INGEZIM AHSV COMPAC PLUS Kit; INGENASA, Madrid, Spain) and positives sera further subjected to capture ELISA using INGEZIM AHS DAS Kit (INGENASA, Madrid, Spain) for antigen detection. Also, spleen samples were collected from 15 donkey carcasses, that died within 24 – 36 hours of unknown causes, for antigen detection using capture ELISA [INGEZIM AHS DAS Kit of INGENASA, Madrid, Spain] and confirmed with conventional PCR using standard procedure (Qiagen; Cod: 210212).

C. Data Analysis

Data entry was performed with Microsoft Excel 2013 (Microsoft Corporation, Redmond, WA, USA). Descriptive statistics comprising percentages were used to indicate the prevalence of AHS in these three regions. The Chi-Square test was used to assess significant levels within factors on seroprevalence rates and odd-ratios were determined for associated risk factors along 95% confidence intervals and statistical significance set at p <0.05 as previously described [29]. All data were computed using IBM SPSS Statistics (ver. 21.0).

D. Ethical Consideration

Permission for the study and ethical approval were obtained from the required local authorities in Cameroon [Regional delegations of Livestock, Fisheries and Animal Industries in the North, Far North and Adamawa Regions and School of Veterinary Medicine and Sciences of the University of Ngaoundéré] before carrying out the study. The purpose of the study was explained (with the assistance of local veterinary practitioners, community leaders and trusted intermediaries) to donkey owner in these study regions. Donkey owners and their animals were used in the study after giving their verbal informed consent.

III. RESULTS

A. Distribution of Donkeys Used in The Study

Of 266 blood samples collected, the highest were from the North (58.65%) followed by Far North (29.70%) and Adamawa (11.65%) regions. Donkeys at least 3 years old (87.97 %) were mostly investigated with female (61.65%) being the commonly encountered animals in the study area (Table 1).

B. Sero-Prevalence of African Horse Sickness in Donkeys in the Northern Regions Of Cameroon

Of the 266 sera screened for anti-AHS antibody detection, 219 were positive for AHSV-specific IgG antibodies corresponding to a sero-prevalence of 82.33% [95%CI: 77.30–86.44] and no antigen detected from the corresponding
whole blood. Locality (region) (p<0.001, χ² = 22.96) and age (p<0.001, χ² = 24.31) significantly influence AHS seroprevalence in this study. Seroprevalence were higher in the North (87.18% [95% CI: 81.03–91.55]) and Far North regions (84.81% [95%CI: 75.30–91.09]) compared to the Adamawa region (51.61% [95%CI: 34.84–68.03]) and also higher in donkeys at least 3 years old [3 to 8 years : (91.08% [95%CI: 85.59–94.61]) and > 8 years: (81.25% [95%CI: 64.69–91.11]) compared to animals less than 3 years old (64.94% [CI: 53.80–74.66]) (Table I).

The Sudano-Sahelian agro-ecological zone [Far North (OR: 5.23) and North (6.38) regions] was significantly (p<0.05) associated with the higher risk of disease dissemination than the high Guinean savannah agro-ecological zone (Adamawa region). Sex was not significant (p>0.05) risk factor of disease dissemination in the present study (Table II). However, donkeys more than 3 years old (89.42% [95%CI: 84.22–93.05]) were at significantly higher risk (p<0.0001, χ² = 22.54, OR: 4.56 [95%CI: 2.36–8.820]) compared to young animals of less than 3 years old (32.14% [95%CI: 27.15–37.13, OR: 0.15]) (Table I). Also, of the 15 spleen samples collected from donkey carcasses in the study, 3 (20.00% [7.05–45.19]) tested positive for antibodies using ELISA Blocking, revealed positivity to antigens with Capture ELISA and also confirmed with conventional PCR.

### Table I: Distribution of Investigated Donkeys by Region, Age Group and Sex in the Study Area

<table>
<thead>
<tr>
<th>Factor / Variable</th>
<th>Number of sample (%)</th>
<th>Region</th>
<th>Number of sample (%)</th>
<th>Region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Adamawa</td>
<td>31 (11.65)</td>
<td>Far-North</td>
</tr>
<tr>
<td>Age group (year)</td>
<td></td>
<td>&lt;3</td>
<td>77 (28.95)</td>
<td>≥8</td>
</tr>
</tbody>
</table>
|                   |                      | [3 – 8] | 157 (59.02) | [95%CI: 27.15–37.13, OR: 0.15]) (Table I). Also, of the 15 spleen samples collected from donkey carcasses in the study, 3 (20.00% [7.05–45.19]) tested positive for antibodies using ELISA Blocking, revealed positivity to antigens with Capture ELISA and also confirmed with conventional PCR.

### Table II: Seroprevalence and Factors Affecting the Dissemination of African Horse Sickness Among Donkeys in the Northern Regions of Cameroon

<table>
<thead>
<tr>
<th>Factor / Variable</th>
<th>Number (positive)</th>
<th>Prevalence % [IC95%]</th>
<th>χ² (p-value)</th>
<th>Odd-ratio [95%CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AD</td>
<td>31</td>
<td>51.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Region</td>
<td>97</td>
<td>84.81</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NO</td>
<td>156</td>
<td>87.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sex</td>
<td>102</td>
<td>79.41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Region</td>
<td>164</td>
<td>84.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Age group (year)</td>
<td>138</td>
<td>77.89-88.95)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;3</td>
<td>(16)</td>
<td>[34.84-68.03]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥8</td>
<td>(67)</td>
<td>[75.30-91.09]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥8</td>
<td>(136)</td>
<td>[81.03-91.55]</td>
</tr>
</tbody>
</table>

AD : Adamawa; FN: Far North; NO : North; ♂: Male; ♀: Female.

The findings of the present study show that African horse sickness (AHS) is highly prevalent in donkeys in the Northern regions of Cameroon. AHS virus-specific antibodies were detected in the donkey sera and no AHS viral antigen was detected in the corresponding whole blood suggesting that the anti-AHS antibody positive donkeys were exposed to the antigen long before the serological screening was done. It is worth noting that antigen detection in blood samples is best at the beginning of the febrile phase [30] and within 90 days of exposure to virus [31], though the antigen has been detected during AHS epizootics and suspected cases in Africa and the viral genome detected up to 120 to 145 days post infection [30], [32]. Also, animals recovering from natural AHSV infection usually develop strong immunity against infection of the same serotypes [33] within 8 to 12 days post infection and vaccination can protect for up to 4 years [3]. High AHS sero-prevalence have been reported in horses (>80%) in parts of Cameroon including the Northern regions [23] and the high detection rate of anti-AHS antibodies recorded in the present study has revealed that AHS is endemic in the country and particularly among domestic equine species. Therefore, the absence of AHS antigens reactions among the positive anti-AHS antibody reactors in this study suggests that the donkeys had developed an active humoral immunity following exposure to the natural disease a long time ago and were no longer actively infected. Though clinical manifestations of AHS were not recorded in the study animals, the detection of circulating anti-AHS antibodies confirmed that the donkeys were convalescing from the natural disease. Also, the anti-AHS antibodies could be lingering in the circulation of fully recovered donkeys while progressively waning in titre.

The AHS sero-prevalence in donkey recorded in the present study was higher than the rates reported in Uganda (73%) [25], Zimbabwe (75%) [34] and Burkina-Faso (72.6%) [26] and lower than 98% in Sudan [35]. The difference may be related to variations in agro-ecological zones and the control strategy of each country. AHS sero-prevalence was higher in the North and Far North regions (Sudano-Sahelian agro-ecological zone with low altitude) compared to the Adamawa region (Guinean savannah agro-ecological zone with moderate altitude), indicating that altitude and climatic conditions influence the distribution of the disease. The finding agrees with previous observations of significantly higher AHS sero-prevalence in Sudano-Sahelian zones in Burkina-Faso [26], high altitude zones in Ethiopia [22] and hot and dry savannah areas in Nigeria [20]. Also, [20], [22] and [36] had reported on influence of the agro-ecological zone and climatic conditions on the survival and multiplication insects and distribution of AHS. The OIE recommends a geographically and climatically sensitive approach to the disease’s surveillance strategy [37]. Outbreaks of AHS following rainy seasons along with increment in the number of vectors and movement of horses from one agro-ecological zone to another have been described [20], [22]. Rainy seasons provide favourable humidity and temperatures and suitable weather conditions of environments which facilitates multiplication of insects as well as propagation and transmission of the AHS virus in...
endemic zones. Low temperatures, long durations of the different life stages can inactive viral replication in insects [38] while high temperatures increase the lifespan of adult insects [39]. Hot environments and periods with heavy rains have been associated with increase reproduction and activities of culicoides [40], [41]. However, decreasing temperatures and increasing rainfalls from the Far-North and North (Sudano-Sahelian agro-ecological zone with low altitude) to Adamawa (Guinean savannah agro-ecological zone with moderate altitude) region was associated with decreasing order of sero-prevalences in this study. The Sudano-Sahelian zone (Far North and North regions) of Cameroon is characterised by sandy environments and can provide suitable habitats for the multiplication of culicoides [41].

In the present study AHS sero-prevalence was determined in both male and female donkeys but sex had no significant influence in the occurrence of AHS. This is well supported by previous findings [22]-[24], [26], [42] that observed no significant variation in sero-positivity in the two sex groups. However, horses kept in modern systems and male horses which are preferentially used by shepherds to accompany cattle herds in search of pastures and during transhumance were at increasing risk of contact with AHS virus than the horses in the traditional systems and female horses, respectively [23]. Unlike horses, there is no preferential use of donkeys based on sex and AHS virus vectors do not have blood meal preference with respect of sex and age.

All age groups showed AHS sero-positivity suggesting that all age groups of donkeys are affected by AHS particularly if the animals had not been previously exposed, recovered and or vaccinated. However, the finding showed significant variation in sero-positivity among the different age groups of the donkeys used in the study. Young animals of less than 3 years old were less at risk compared to older horses. Despite the possible loss of maternal immunity, some young colt could have had no contact with the virus while older donkeys had more opportunities to be exposed to the virus. Similar observations were reported by [5] in Senegal and [23] in Cameroon where old animals were more likely come into contact with the virus.

The absence of a vaccination program against AHS in Cameroon coupled with the fact that it is a vector-borne disease, testifies not only to natural infection with the circulation of wild strains with naturally attenuated virulence or not very virulent, but also to the presence and activity of vectors. AHS sero-positivity is high among horses [23]; and donkeys as well as other livestock occupy the same microenvironment in agro-pastoral communities of Cameroon including those in the Northern regions. Orbivirus which possess nine serotypes [2] with 84 strains [1] is the cause of AHS in all equine species [3], [4], solipseds [4] and accidentally carnivores [5]-[7], [9]. There has been identification of undeclared AHS virus (strains and serotypes) in equine in central Africa [43] and following sporadic outbreaks of AHS in horses and donkeys in North and Far North regions of Cameroon [44]. Though the AHS viral strain(s) and serotype(s) circulating in equines in Cameroon is not clear, a state of endemic stability to the serotype(s) could have developed based on one or more of the following (i) repeated infections of a naive population by wild strain(s) for several years, (ii) infection of the equidae at an early age (and production of natural antibodies) while still possessing partially or totally antibodies inherited from maternal colostrum and (iii) existence of cross reactions with one or more unidentified orbiviruses [45]. The finding of the present study and its contextual comparison with previous results of AHS in horses in parts of Cameroon [23], affirms that AHS is highly endemic in the agro ecological zones of Cameroon (lowland, midland and highland) which are major contributors (over 95%) of the national domestic equine (horses and donkeys) herds [27].

V. CONCLUSION

This study highlights that African Horse Sickness virus exists in Cameroon and there is high anti-AHS antibodies seropositivity in donkeys in the country. Though agro-ecological and age are major factors influencing the sero-prevalence of AHS in donkeys, donkeys play major roles in its maintenance and continuous surveillance is important for decision making to reduce the burden of African Horse sickness in animal health in Cameroon.

ACKNOWLEDGMENT

The authors are grateful to the staff of the Delegations of Livestock, Fisheries and Animal Industries in the Far North, North and Adamawa Regions for the technical and logistical supports and to donkey owners in the study regions for allowing the collection of samples and their generous cooperation.

CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

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