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# Sero-prevalence and risk factors of African Horse Sickness among donkeys in a highland area of Kenya

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**A study was conducted to determine the prevalence and risk factors for African Horse Sickness (AHS) in donkeys in the highland area of Kiambu County, Kenya. Data on the risk factors was collected using semi-structured questionnaires administered to 146 donkey owners while blood samples collected from 398 donkeys both during the rainy and dry seasons. Antibodies against AHS were detected using competitive antibody Enzyme Linked Immuno – Sorbent Assay (c-ELISA). The estimated sero-prevalence of AHS in donkeys was 35.2 and 27.6% during the rainy and the dry seasons, respectively. The sero-prevalence of AHS in the donkeys that were re-examined during both seasons decreased from 60 to 20% indicating a waning immunity. Age of the donkey (P= 0.02) and presence of water streams (P= 0.03) were significant risk factors of AHS among donkeys. Three seropositive donkeys also showed pulmonary clinical signs of AHS. There was poor agreement (k =0.05) between the c-ELISA and clinical diagnosis of AHS. In conclusion, AHS in donkeys is endemic in the highland area of Kenya. This calls for increased adoption of preventive measures against the disease and justifies the need for future research on clinical and epidemiological patterns of AHS in donkeys in other highland areas.**

**Key words:** African horse sickness, donkeys, highland area, prevalence, risk factors.

## INTRODUCTION

Donkeys are commonly regarded as hardy animals (Croxton, 1993) although diseases have often posed an enormous threat to their effective use for draught purposes (Mellor and Hamblin, 2008). African Horse Sickness (AHS) is an arthropod-borne viral disease of horses, mules, donkeys and zebra, caused by a double-stranded RNA virus of the same name, the African Horse Sickness Virus (AHSV). The disease is endemic in equines in sub-Saharan Africa (OIE, 2017; Tomori et al., 1990). There are nine distinct identified serotypes of AHSV (Mellor and Mertens, 2004). Donkeys are infected with AHSV serotype 4 (Hamblin et al., 1998) as well as serotype 6 (Zeleke et al., 2003).

*Culicoides,* the main biological vectors involved in transmission of AHSV in Africa, are tiny biting midges about 1-3 mm in length. Other blood sucking arthropods such as mosquitoes *Stomoxys* and *Tabanus* which transmit many blood borne pathogens may act as

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potential vectors (Sumbria and Singla, 2017; Carpenter et al., 2017; Mohammed et al., 2017). *Culicoides* breed in wet soil during the rainy seasons and in organic matter such as animal dung (Meiswinkel et al., 1994). They also breed in pools, streams, marshy areas, swamps, beaches, tree holes, irrigation pipe leaks, saturated soil, animal dung, rotting fruit and other vegetation (Mellor et al., 2000). While their numbers increase with moisture in the environment during the wet season, irrigation has been shown to maintain high numbers of *Culicoides* during the dry season (Meiswinkel, 1998). In South Africa, major epidemics of AHS occur every 5 years during the *El Nino* phenomenon suggesting a strong link between the timing of these epidemics and the high humidity during the *El Nino* (Baylis et al., 1999; Carpenter et al., 2017). High wind speeds which aid vector dispersal have also been associated with increased AHS prevalence (Sellers and Mellor, 1993).

Most susceptible animals are infected between sunset and sunrise, which is the period during when the *Culicoides* are most active (Coetzer and Guthrie, 2004). The rate of virogenesis within the vector is faster at higher temperatures due to increased feeding frequency enhancing the probability of the viral transmission (Wellby et al., 1996).

The AHSV can cause four forms of disease namely pulmonary form, cardiac form, mixed form and horse sickness fever (Murcia et al., 2009). The disease is fatal in horses. Donkeys exhibit horse sickness fever, involving mild to moderate fever and oedema of the supra-orbital fossae with no reported deaths (Coetzer and Erasmus, 1994). Zebras are thought to be the natural reservoir host in many African countries (Carpenter et al., 2017). The virus can infect dogs and camels experimentally although these species does not play a role in the natural spread or maintenance of AHSV (Alexander et al., 1995).

AHSV has been isolated in Kenya in horses (Davies et al., 1993). The disease is endemic and notifiable in this species in Kenya (Animal diseases Act CAP 364, 2012), but has not been declared present in donkeys because information on the confirmatory diagnosis of AHS in donkeys in Kenya has not been reported. Some veterinarians in Kenya have previously observed clinical signs that were suggestive of AHS in donkeys (Unpublished clinical records of diseases in donkeys by the government veterinary officer Kiambu County). In addition, there is no information concerning the prevalence of AHSV antibodies among the donkey populations in Kenya. AHS may be confirmed either by isolation of the virus or identification of AHSV antigens and antibodies using serological techniques which include ELISAs, complement fixation test and immunoblotting (Mellor and Hamblin, 2004; OIE, 2017). In ELISA method, for example, known antigen is used to detect circulating antibodies in the donkeys' serum samples. The current study was designed to measure the prevalence and determine the risk factors of AHS in donkeys in the Central highland area of Kenya in Kiambu County. The findings would provide evidence for declaration of presence AHS in donkeys in Kenya and will be useful for designing preventive health programs such as vaccination and vector control for optimization of health and welfare of donkeys.

## MATERIALS AND METHODS

### Description of the study area

The study was carried out in the highland areas of Kiambu County in Lari and Limuru sub-counties where donkeys are many. Kiambu County is located in the central region of Kenya and borders Nairobi and Kajiado Counties to the South, Machakos to the East, Murang'a to the North and North East, Nyandarua to the North West, and Nakuru to the West. It lies between latitudes 00 25' and 10 20' South of the Equator and Longitude 360 31' and 370 15' East (KCIDP, 2018).

The County is divided into four broad topographical zones; upper highland, lower highland, upper midland and lower midland zone lying at an altitude of between 1,200 - 2,550 m above sea level. Lari Sub-county is located in the upper highland areas with altitudes of 1,800-2,550 m above sea level and tapers from the Aberdare ranges while Limuru Sub-county is located in the lower highland zones in altitudes of 1,500-1,800 m above sea level. The county is characterized by steep slopes especially in areas bordering the Aberdare ranges to hills, plateaus and high elevation plains. Some parts are also covered by forests such as Kinale and Kiriita in Lari Sub-county. Donkeys freely associate with Zebras while grazing in these forests. The soils are suitable for farming of cash crops such as tea, coffee as well as a variety of food crops. The main economic activity is agriculture which is however constrained by the small land sizes of 1ha or less (KCIDP, 2018). The rainfall pattern in the region is bimodal ranging from 750 to 1300 mm annually. The long rains occur between March to May, followed by a dry season from June to September. The short rains then follow from October to November and another dry season from December to February. Rainfall distribution is reliable and has largely influenced agricultural activities in the area. Temperatures range from an average of 20.4°C in March/April to 12.5°C in July/August in the upland zone. Lari sub-county is subdivided into 9 locations and 23 sublocations, while Limuru sub-county is divided into 5 locations and 19 sublocations. The locations which were sampled from Lari sub-county were Kambaa, Kirenga and Mukeu while those sampled from Limuru sub-county included Ngecha, Rironi and Rwamburi. The estimated donkey population in the study area was 6,825 (CBS, 2010). Other equines such as horses and zebras as also present in the county (KCIDP, 2018) although their actual numbers are missing since they are not considered domestic livestock. The locations where the study was carried out are indicated in Figure 1.

### Sampling and sample size determination

The human population of interest was households owning donkeys in Limuru and Lari sub-counties. Six locations within these two sub-counties were selected by stratified random sampling, with three locations in each of the two divisions selected (Figure 1). A list of households owning donkeys in each study location was generated

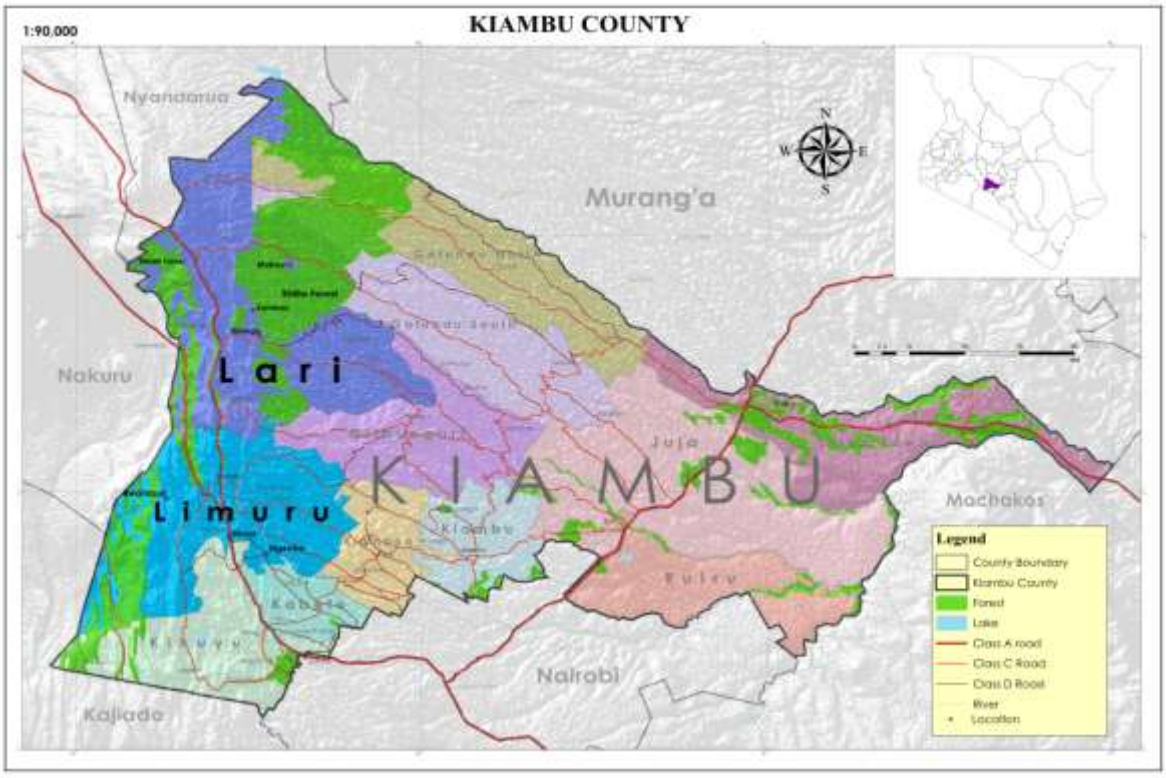


**Figure 1.** Map of Kiambu County showing Lari and Limuru Sub-counties and the study sites (in bold). Source: Orbital Geospatial services www.orbital.co.ke.

with the assistance of the local chiefs and administrators. The households were then re-ordered by random selection and visited in order until the required number of donkeys to be sampled was achieved. Prior to data collection, the study objectives were explained to the donkey owners and verbal consent to participate in the study was sought and obtained. They also gave permission to bleed their donkeys. Assuming a prevalence of 50% for AHS, the number of donkeys to be sampled for sero-surveillance was computed according to a formula by Martin et al. (1987): $n=Z_{\alpha}^{2}pq/L^{2}$. Where, n was the required sample size, $Z_{\alpha}$ was1.96, the standard normal deviate at 5% level of significance, p was the estimated prevalence at 0.5 while q was 1-p, and L was the precision of the estimate and was set at 5%. The required sample size n was 384 donkeys. The area chiefs and their assistants were engaged to mobilize donkey owners and provide the list of households with donkeys. The chiefs assisted in identifying the village guides to direct the study team to the selected households.

## Ethical approval

Prior to conducting the research, ethical clearance was granted by the University of Nairobi; Faculty of Veterinary Medicine Biosafety, animal use and ethics committee *REF: FVM BAUEC/2010/165.*

## Data collection

Sampling of donkeys at the homestead level for sero-surveillance of AHS was done during the rainy season (April and May) and during the dry season (August and September). This sampling strategy was adopted since temperature and moisture have been shown to affect the prevalence of AHS (Ventor et al., 2000). It was hypothesized that the vector population (*Culicoides*) would be different between the two seasons.

Prior to bleeding, a physical examination of the donkeys was performed. The body condition of the donkeys was scored, based on muscle and fat distribution and prominence of the spine, hips and ribs, on a scale of 1-5; with 1=thin, 2=Moderate, 3=ideal, 4=fat and 5=Obese (Donkey Sanctuary, 2014). The age of the donkey was determined by dentition based on eruption of and shape of the permanent incisors as described by Muylle et al. (1999). Donkeys were manually restrained by use of a halter and blood drawn by venopuncture of the jugular vein, using gauge 18 venoject needles. Blood was collected into 10 ml vacutainer tubes without anti-coagulant. The blood samples were placed in a cool box, transported to the laboratory and stored at room temperature (20-24°C) overnight for serum separation. The clotted blood was then centrifuged at 3000 revolutions per minute for 5 min, after which the serum was harvested using disposable pipettes and stored in 10 ml cryo-vials at -20°C until laboratory testing.

146 donkey owners were also interviewed using a structured questionnaire to determine the associated risk factors. Data collected included the respondents' location and contacts, stabling regime of the donkeys, the uses of the donkeys, disease control strategies, as well as presence or absence of the breeding sites for the AHS vectors such as soil types and their drainage, marshy areas, nearby water streams, accumulated animal dung or

decomposing organic waste, use of irrigation or any other potential vector breeding sites (Meiswinkel et al., 1994; and Mellor et al., 2000). In case sick donkeys were sampled, their clinical signs were compared against commonly used clinical signs to diagnose AHS in equines. These signs included high fever, depression, conjunctivitis, oedema of the head, dyspnea, and respiratory failure (Coetzer and Erasmus, 1994).

### Laboratory analysis

Competitive Antibody Enzyme Linked Immuno-Sorbent Assay (c-ELISA) was conducted using a kit from CVRL (Central Veterinary Research Laboratories, Dubai) using the method previously described by Hamblin et al. (1990). The kit used in this study was not specific for any of the various serotypes of AHS.

### Data handling and analysis

Data were entered into Microsoft Excel (Microsoft Corporation) spreadsheets, 2007 version. The prevalence of AHS in donkeys was calculated as simple proportions for both divisions and overall study area, by dividing the number of seropositive samples by the total number of samples tested. The data on the potential risk factors for AHS were entered into Microsoft excel (Microsoft Corporation) spreadsheets, and then exported into StataR version 11 for generation of descriptive statistics. A mixed models logistic regression analysis was conducted having adjusted for clustering effects within the homesteads. The data structure was multilevel (hierarchical) from sub-county, location, household to donkey.

Sub-county and location were controlled for in univariate analysis and the corresponding likelihood ratio tests were carried out in order to obtain the probabilities of the individual variables. Associations were considered significant at a P value of ≤ 0.2. Forward fitting of a logistic model was then done at multivariate analysis stage using the variables that were considered statistically significant in explaining the outcome from the univariate analysis where variables giving a P value of 0.05 or less were retained in the final model. The Intra-cluster Correlation (ICC) was evaluated at each stage of the analysis. The magnitude of the effect of clustering on variance (estimate) depends on the size of the clusters and the ICC which is the correlation between observations within the same household. A low ICC meant that most of the variation was within the groups (hence little clustering), while a high ICC meant that the variation within a group was small relative to that between groups (Dohoo et al., 2003). The logistic regression equation with random effects was:

$$\text{Logit}(p_i) = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} \ldots\ldots \beta_k x_{ki} + u_{household\ (i)},$$

where Logit ($p_i$) is the logit transform, βo is the intercept, $\beta_1$ and $\beta_2$ represents the regression coefficients associated with fixed effects $x_1$ and $x_2$ independent variables for the $i^{th}$ donkey (suggested risk factors), and $\boldsymbol{u}_{\text{household (i)}}$ represents the random effect of the household (which contains donkey i).

The importance of the risk factors in the donkey population were estimated using the population attributable fraction (PAF) according to the method described by Dohoo et al. (2003) which was PAF = Prevalence ÷ PAR (Population Attributable Rate).

The level of agreement between Competitive antibody-ELISA and the commonly practiced clinical diagnostic methods was examined. This was done using the Kappa statistic for agreement procedure which tests the agreements between two diagnostic tests (Dohoo et al., 2003).

## RESULTS

The required sample size of 384 donkeys was exceeded by sampling 398 donkeys. Equal number of donkeys (199) were sampled in the wet and dry seasons. Questionnaire data of risk factors was collected from 146 households in the first sampling period during the wet season. The average number of donkeys per household was 1.4 (199/146). More male donkeys (62.6%; 125/199) were found compared to females (37.4%; 74/199). The average age of the donkeys was 9 years (range 1 to 30 years). Majority (87%) of the donkeys were kept for domestic purposes with 13% for commercial purposes. The donkeys were acquired from the livestock markets (61.6%), from neighbors 23.7% or through farm breeding 14.6%.

In the second sampling period during the dry season, a different random sample of donkeys was bled in the study locations except Kambaa location where the 30 donkeys sampled in the wet season were re-sampled in the dry season in order to study changes in their disease status.

Three donkeys with poor temperament were replaced as they could not be safely restrained for bleeding. Replacement was also done for four donkeys whose owners denied consent for participation in the questionnaire interviews and for blood sampling giving a household response rate of 97.3% (146/150) for the questionnaire.

The most commonly observed potential breeding sites by the vector of AHS in the surveyed households sites were accumulated dung (38.3%), ponds (28.1%), water streams (23.3%), bushy area (21.2%), stagnant water (20.5%) and a nearby forest (15.8%). All the surveyed households used rain water for farming, and an additional four of these farms supplemented rain water with irrigation during the dry seasons. About 83%; (121/146) of the households were located in farms that had soils which drained rain water in less than 3 days.

Restlessness, stamping of feet, rubbing bodies against rough surfaces was observed in 75.3% (150/199) of the donkeys by their owners. These signs were attributed to domestic flies, mosquitoes, biting flies, fruit flies, ants, fleas and other unknown insects. Human insect bites by mostly mosquitoes (84.8% (124/146)) reportedly increased during and immediately after the rainy season compared to the dry season. About 12% (17/146) of households surveyed used acaricides in their farms for spraying all livestock. On the other hand 35% (70/199) of the surveyed donkeys were de-wormed and vaccinated against tetanus but not against AHS.

On housing and stabling regime, 47% (93/199) of the donkeys were housed, 39.3% (78/199) were tethered near the homestead, 7.7% (15/199) were let free within the homestead, 5.5% (11/199) were kept in the forest and only 0.9% (2/199) were tethered near a pond. During the

rainy season, 70/199 donkeys tested positive for AHS while in the dry season 55/199 donkey tested positive giving a prevalence of 35.2%; (95% CI; 28.5, 41.8) and 27.6% (95% CI; 21.4, 33.9) respectively. The difference in prevalence between the two seasons was however not significant.

According to the mixed model analysis applied to the data due to the clustering effect, several risk factors were considered and assessed. On univariate analysis; age category, stream of water, source of donkey, donkey use, vaccination status and housing were considered statistically significant and hence included in the multivariate analysis. Multivariate analysis of the data revealed that age category (P= 0.02) and stream of water (P= 0.03) were the only statistically significant risk factors for AHS in donkeys. These two variables were included in the final model as significant explanatory variables for AHS the central highland areas of Kenya. The final model was:

$$Logit_{AHS} = -1.4 + 0.66_{Age\ category1i} - 1.76_{Water\ stream2i} + u_{household\ (i)},$$

The data had an ICC of 0.7 meaning the data was highly clustered within the households implying that donkeys from the same household had similar ELISA results. The estimated population attributable fraction (PAF) for the presence of a stream of water was 22% indicating that 22% of the AHS antibodies in the donkey population of Kiambu County were due to the donkeys grazing near the water streams. Similarly, the PAF estimates for donkeys less than 9 years old and those above 12 years were 33 and 30%, respectively. There was a poor agreement (kappa, $k$ = 0.05) between Ab- ELISA test results and the clinical signs of AHS of swollen head and congested conjuctival mucosae, the commonly used clinical diagnostic tests of AHS in donkeys. Only three donkeys which tested positive for AHS displayed clinical signs.

## DISCUSSION

The current study was conducted to estimate the prevalence of AHS in donkeys as well as to determine the risk factors associated with the disease. Competitive Ab ELISA, with specificity and sensitivity both of 96%, was used to detect antibodies against AHS in serum samples collected from donkeys. The results demonstrated an AHS prevalence of 35.2% in the donkey population during the heavy rains in April to May, and 27.6% in the dry season in August and September, 2010. This prevalence was attributed to exposure of AHSV because the sampled donkeys were never vaccinated against AHS. African Horse Sickness sero-positivity in donkeys was also observed in all six locations studied. This could indicate an endemic nature of the disease in the central highland region.

The sero-prevalence estimates of AHS in donkeys in this study was similar to that stated for Ethiopia at 36.4% (Ende et al., 2013) with similarities in climatic conditions favoring vector presence in the two countries. A higher prevalence (75%) was reported in Zimbabwe (Gordon et al., 2017) in donkeys during the rainy season but this was attributed to night transmission where the donkeys were rarely stabled coinciding with the period of high vector activity (Gordon et al., 2015); Coetzer and Guthrie, 2004).

While the drop in prevalence levels (60-20%) recorded in Kambaa location when donkeys were resampled indicated waning immunity. The overall drop in prevalence of AHS in donkeys during the dry season was attributed to the decrease in the vector numbers (Meiswinkel, 1998). Mosquitoes were identified as the most common human biting insects present in the region occurring mostly (79.3%) during the rainy season. Other unknown biting insects were also present in the region but they represented only 11.4%. Since the conditions favoring presence of mosquitoes were similar to those for *Culicoides* (Tomori et al., 1990), it was assumed that the latter were present but were either unidentified due to their small size (1-3 mm in length), or they were part of the unknown biting insects. Further entomological studies, however, need to be carried out in order to confirm the presence of *Culicoides* in Lari and Limuru Divisions, and to identify the unknown biting flies. Studies should also be carried out to confirm or dispute the role of mosquitoes in the transmission of AHS in donkeys in the study area and other parts of Kenya.

Use of insecticides did not appear to influence the sero-positivity of the donkeys. Although insecticides were shown to control the *Culicoides* through application to their breeding sites, donkey housing or applied to animals as repellants (Carpenter et al., 2008). Other control methods for *Culicoides* included removal of their breeding sites or sheltering animals in insect proof housing (Lincoln et al., 2015). This was shown to reduce the contact between the host and the vector when the vectors are most active (Williams et al., 1993; Coetzer and Guthrie, 2004). The kind of housing observed in 47% of the sampled donkeys' homesteads comprised of a roof without walls which was not protective against the *Culicoides*. The rest (53%) were either tethered or let free around the homestead and in communal grazing lands.

Majority of the sampled donkeys were older (9-12 years on average) because most owners preferred to purchase mature donkeys from the market. Since Limuru livestock market was the most preferred site it was probable that these donkeys could have been exposed prior to purchase. Occurrence of AHS in donkeys within this bracket could therefore be as a result of sampling more donkeys in this age bracket and not a causal risk factor

for AHS even with a significant result (P = 0.02).

Presence of a stream of water had a plausible effect on sero-positivity for AHS as it was an ideal site for vector breeding and therefore increased vector population. It was also a vehicle to transport the vector in the immature stages (larval). The study revealed a direct relationship between AHS sero-positivity and donkeys grazing near streams of water (P = 0.03). More risk factors ought to have explained the disease in donkeys hence creating a gap for more risk factors studies. The high ICC of 0.7 showed that AHS was highly clustered within households hence most of the variation of the outcome was between households (as opposed to that within households). This was a very important measure especially for researches wishing to compute sample sizes for AHS in future in order to conduct epidemiological studies.

Only a small proportion (4.28%) of the ELISA positive donkeys screened showed obvious clinical signs in the wet season. This finding differs with Coetzer and Guthrie (2004) who stated that donkeys were often affected by the AHS fever form of AHS. During the dry season sampling period there were no clinical cases reported. This finding concurs with that of Williams et al. (1993) who stated that AHS occurred seasonally with outbreaks being associated with conditions favoring breeding of the vector, which was mostly during the wet season. The poor agreement (k =0.05) between the c-ELISA and clinical diagnosis of AHS indicated that absence of clinical signs did not imply lack of exposure to AHSV. Use of serology was therefore a better diagnostic method of testing donkeys for AHS. It is however, relatively expensive in terms of time (24 h) and money ($ 8) to test one sample. Further, the availability of testing kits was a challenge as they had to be imported. It was recommended that the Veterinary Laboratories in Kenya should stock the kits and train staff on testing donkey sera suspected of AHS. More studies needed to be carried out to identify the antigenic serotypes responsible for causing clinical disease in donkeys to ensure that vaccine production was tailored to provide specific immunity against the disease.

This study represents the first report of AHS in donkeys documented in Kenya. The high prevalence of AHS in this region calls for attention by Animal Health stakeholders, and prompt action to reduce this level through vaccination of donkeys and targeted vector control especially in the wet season.

## Conclusions

AHS is endemic in donkeys in the highland area of Kenya. Its prevalence was 35.2% in the rainy season and 27.6% in the dry season. Donkeys, in addition to horses, also exhibit clinical signs of AHS although clinical diagnosis was a poor indicator of exposure to AHSV. Age of the donkeys (9-12 years) and presence of a nearby water stream were the significant risk factors for AHS in this environment.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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