

Evaluating African horse sickness virus in horses and field-caught *Culicoides* biting midges on the East Rand, Gauteng Province, South Africa

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Summary

A prospective study was undertaken during 2013 and 2014, to determine the prevalence of African horse sickness virus (AHSV) in *Culicoides* midges and the incidence of infection caused by the virus in 28 vaccinated resident horses on two equine establishments on the East Rand, Gauteng Province, South Africa. Field caught *Culicoides* midges together with whole blood samples from participating horses were collected every two weeks at each establishment. *Culicoides* midges and blood samples were tested for the presence of AHSV RNA by real-time quantitative reverse transcription polymerase chain reaction. Nine immunised horses became infected with AHSV during the study period, although infections were subclinical. African horse sickness virus was also identified from a field-collected midge pool. The observations recapitulate previously published data in another setting, where further investigation is warranted to determine what role subclinical infection plays in the diseases epidemiology.

Virus della peste equina (AHSV) nei cavalli e nei *Culicoides* nell'East Rand, nella provincia di Gauteng, Sud Africa

Parole chiave

African horse sickness virus,
Cavalli,
Culicoides,
Subclinico,
Sud-Africa.

Riassunto

Tra il 2013 e il 2014 è stato condotto uno studio per determinare la prevalenza del virus della peste equina (AHSV) nei *Culicoides* e l'incidenza dell'infezione causata dal virus in 28 cavalli di due stabilimenti equini dell'East Rand, nella provincia di Gauteng, Sud Africa. Ogni due settimane, in ciascun stabilimento, sono stati catturati i *Culicoides* e raccolti i campioni di sangue dai cavalli; è stata testata la presenza di AHSV RNA mediante *PCR real-time quantitative reverse transcription*. Pur trattandosi di infezioni subcliniche, durante il periodo di studio sono stati infettati da AHSV nove cavalli immunizzati. Il virus della peste equina è stato anche identificato in un gruppo di *Culicoides* catturati sul campo. Le osservazioni ricapitolano dati pubblicati in precedenza per un contesto diverso, in cui sono state condotte ulteriori indagini per determinare quale ruolo svolge l'infezione subclinica nell'epidemiologia delle malattie.

African horse sickness virus (AHSV) has an intense and negative impact in equine veterinary science both within South Africa and internationally. This non-contagious arthropod borne disease is endemic to sub-Saharan Africa, with periodic outbreaks having occurred in Turkey, Cyprus, Lebanon, Syria, Iraq, Afghanistan, Iran, Pakistan, India, Egypt, Morocco, Spain and Portugal (Lubroth 1988, Mellor and Hamblin 2004). African horse sickness (AHS) having a mortality rate of up to 90% with the potential for rapid international spread, further illustrates its profound economic importance (Guthrie and Quan 2009). Transmission of the virus occurs via the bite of an infected haematophagous *Culicoides* midge vector, of which the most significant is *Culicoides (Avaritia) imicola* Kieffer, followed closely by *Culicoides (Avaritia) bolitinos* Meiswinkel (Meiswinkel *et al.* 2000). Throughout most of the endemic range within South Africa, the climate is suitable for adult *Culicoides* midges to remain active throughout the year, therefore suggesting a continuous transmission cycle is possible, where the virus may circulate over-winter either in subclinically infected hosts like donkeys and/or zebras as well as in *Culicoides* midges and associated equine populations (Venter *et al.* 2014). Since there is no curative treatment for AHS, vaccination and vector control are performed to prevent and control this disease limiting horse / vector exposure thus reducing viral infection in susceptible horses (Guthrie and Quan 2009).

During 2013 and 2014, samples were collected from two equine establishments (Establishment A and B) to determine the prevalence of AHSV in *Culicoides* midges and the incidence of infection caused by the virus in 28 resident horses. The two establishments were located on the East Rand, Gauteng Province, South Africa, approximately 20 km from each other, where the East Rand is a summer rainfall area with dry winters and occasional frost, resident to an abundance of smallholder equestrian facilities. The selection of each establishment was closely linked to; management, stabling facilities, vector control and vaccination procedures.

Establishment A was selected as the control establishment, where good record keeping and reporting systems were in place to detect any abnormality, horse's rectal temperatures were recorded twice daily, insecticides were applied regularly and insect proof accommodation was made priority in an attempt to lower midge numbers in stable blocks; together with eliminating any insect breeding sites through general establishment cleanliness. Vaccination protocols followed were those recommended by the vaccine manufacturer Onderstepoort Biological Products SOC (Ltd) (OBP) and facility veterinarians.

In contrast, Establishment B was an old farming

enterprise with a poor infrastructure and dilapidated stabling facilities. Preventative measures were haphazard and the equines lived outside, free to roam the farm including low lying water catchment areas providing an adequate breeding habitat for the *Culicoides*. Vaccination was not regularly part of the management regime in previous AHS seasons, however, vaccination was administered by the owner prior to the start of the study using the OBP polyvalent live attenuated vaccine as was used at Establishment A.

During the study the 28 horses, 15 from Establishment A and 13 from Establishment B, were clinically examined for any signs of AHS; rectal temperatures were recorded by digital thermometer and whole blood was drawn by jugular venepuncture in ethylene diamine tetra-acetic acid (EDTA) Vacutainer® tubes from each horse on a two-weekly basis during the 2013-2014 AHS season (December-May). Blood was stored at 4 °C until analysed for the presence of AHSV by real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR) as previously published by Quan and co-workers (Quan *et al.* 2010). All Blood samples were classified as positive with a 95% limit of detection, when the cycle threshold (Ct) value of the samples was < 37.14.

A total of nine (9/28) immunised horses showed RNAemia during the study where all horses were subclinical and showed no change to their habitus and had no rise in rectal temperature, appearing clinically normal on the day of sampling. As the assay does not distinguish between vaccine and wild virus, it was important to consider the date of vaccination in relation to the start of the study. We found that three (3/28) horses showed RNAemia at the start of the study where we draw the inference that these three could be vaccine reactions and not natural field infection as vaccination was performed 2-3 weeks earlier. The remaining six horses (6/28; one from Establishment A and five from Establishment B) showing RNAemia during the course of the study were negative for AHSV RNA at the start, which implied these six were naturally infected with wild virus under field conditions during the AHS season.

In order to further evaluate the prevalence of AHSV in the immediate area, *Culicoides* midges were collected using the 220 V Onderstepoort downdraft suction light traps operating with an 8 W UV-light tube (ARC-Institute of Agricultural Engineering, South Africa) as previously described by Venter and colleagues (Venter *et al.* 2009). Insect collections were stored in 70% ethanol at 4 °C until midge separation and identification, where collections were then differentiated and pooled into groups of 200 according to collection dates, relevant species, gender and parity status. Pools contained only

C. imicola and *C. bolitinos* of which are the most significant vectors.

Upon analysis of the field-caught *Culicoides* midge collections, the total sum of *C. imicola* and *C. bolitinos* midges trapped at both establishments during the collection period was 11,157 sorted into 91 like pools. In an attempt to understand the seasonal disease prevalence on both participating establishments; pooled midge collections were evaluated for the AHSV RNA by using the same RT-qPCR as used for evaluating the blood samples, where samples were considered positive when the Ct value was < 40 (Guthrie *et al.* 2013).

Of the 91 pools collected (55 from Establishment A and 36 from Establishment B), only 1 parous pool (1/91) from Establishment B revealed a positive result by RT-qPCR, for the presence of AHSV RNA with an observed field infection prevalence of 2.7%. We cannot however determine if this was due to a single positive midge or multiple midge infections. Notably, AHSV could be identified from a field-collected midge pool suggesting that vector competent populations are present.

In this study the number of midges collected was significantly lower than those reported in larger studies by Scheffer and co-workers (Scheffer *et al.* 2011). The prevalence of the disease is dependent on a build-up of virus in midge populations which must first reach a critical level, thereafter spill over will occur into associated equine populations (Venter *et al.* 2006). This could be one likely explanation for the low infection prevalence detected in field-collected midges on each establishment. Another likely explanation to the low prevalence of infection detected in the midges collected from each establishment was due to the lower number of AHS cases reported around the study area during the study period. This lower number of viraemic horses will be directly proportional to the lower field infection prevalence found in field-collected midges, subsequently reducing amplification of the virus in vector populations (Venter *et al.* 1997, Venter *et al.* 2006).

Although midge populations were reduced in number, AHSV was present in the area where subclinical infection in horses was evident producing a detectable viraemia using PCR. Weyer and colleagues (Weyer *et al.* 2013) reported that horses that had been vaccinated, as in the case of Establishment A and B, could still become infected with the AHSV subclinically, and speculated that they could be a source of virus for infecting midges. In a study by Mellor and colleagues (Mellor *et al.* 1975), a minimum experimental viral titre of 10^4 - $10^{4.5}$ MID₅₀ / 0.02 ml blood, was sufficient for laboratory colonies of *C. sonorensis* to become infected with AHSV when a blood

meal was ingested. Following a 13-day incubation period, these midges were then able to transmit the virus. Furthermore, the rate of transmission of Bluetongue virus (BTV) a closely related orbivirus, was evaluated by Baylis and colleagues (Baylis *et al.* 2008), with the same species of *Culicoides* as used by Mellor and colleagues (Mellor *et al.* 1975), verifying experimentally that a single midge with a viral titre > $10^{2.9}$ TCID₅₀ / ml could reliably transmit the BTV to susceptible sheep. Whether these findings can be extrapolated to AHSV in the African context requires further investigation as no threshold value has yet been determined for a subclinically infected horse to infect a smaller midge species such as *C. imicola* or *C. bolitinos* (Weyer *et al.* 2013).

We hypothesised that the incidence of AHS would be far higher on the poorly managed horse Establishment B. Our objective being to compare and contrast disease incidence on a well and poorly managed horse establishment. The management of Establishment B however, unexpectedly improved their prophylactic measures by vaccinating. This hindered our objective as vaccinating would prevent virus amplification in the horses, reducing the potential for secondary vector spill over and build-up of virus in midges on Establishment B as it would on Establishment A (Bird *et al.* 2011). The incidence of AHS on Establishment B was low during the study where mortality rates had been high in the past as attested by the attendant veterinarian. Vaccination however could not be the sole constituent when evaluating the reduction in the number of clinical cases evident during the study period. We need to emphasize and evaluate the several confounding factors at play, including the continuous herd exposure to the virus in previous seasons, where it has been shown that natural infection of horses with wild-type virus will induce a broader and stronger cross-reactive immunity than that observed with the live-attenuated vaccine (Blackburn and Swanepoel 1988). This could incorporate greater herd immunity where no clinical manifestation of disease was evident during the study and therefore this together with vaccination, increased the probability of protection against AHS, presenting with lower disease incidence.

Although the incidence of infection was low during the study, the observations confirm that on both establishments immunised horses became infected subclinically with AHS. Although subclinical cases found are substantially fewer than the clinical cases, they are still a concern in terms of spread of disease (Grewar *et al.* 2013). How such cases influence viral transmission warrants further investigation, where answering how the virus alters its capacity to infect horses either clinically or subclinically can be used to decrease the dissemination of this disease, all the more with evidence of genome re-assortment and

the reversion to virulence of live attenuated AHS vaccine viruses (Weyer et al. 2017).

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Statement of animal rights

Materials used in these experiments posed no health risk to researchers and no vertebrate animals were harmed. Research was conducted in accordance with the institutional Animal Ethics Committee guidelines. Project approval number V066-13.

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