

## 4. DISCUSSION AND CONCLUSION

### 4.1. Discussion

Environmental conditions tested in this study appear to have had an influence on the occurrence of *E. z. hartmannae*, a possible cycling host for AHSV and the *Culicoides* midge vector although not in the manner expected.

*E. z. hartmannae* were found to roam the entire south-western Khomas Region, but was more concentrated in the drier south-west than the wetter north-east. They migrate towards the north-eastern high ground during droughts, presumably along ephemeral river beds and may cover vast areas. This vast range has implications for the control of the disease. An infected zebra as cycling host can potentially carry the virus from a distant infection source and expose horse populations many kilometres away, such that the management practices of one horse owner may have an effect on another's stock at a considerable distance away.

*Culicoides* midges were collected at all sites along the study transect, across a rainfall gradient in the south-western Khomas Region, even during winter. The *Culicoides* midge communities included species that are known AHSV-vectors and three more species suspected to be AHSV-vectors (Nevill *et al.*, 1992). These communities in the south-western Khomas Region are therefore considered capable of AHSV transmission. In future studies it could be interesting to investigate whether or not there is a lag-time response of *Culicoides* midges following the cyclic pattern of the annual rainfall illustrated in Fig. 3.5.

The occurrence of AHS in horses, as standardised by converting cases of AHS to AHS incidence proportion, occurred mostly in the mid-rainfall zones, rather than where it was expected, at the highest rainfall areas. However, as expected, in the lowest rainfall zones, below 150 mm/a, no cases of AHS were reported. More areas must be sampled to test the validity of this observation. However, should further sampling still reveal this pattern, future research may point to other variables which affect the occurrence of AHS incidence proportion.

The abundance of *Culicoides* midges collected at sites along the rainfall gradient did not show a clear relationship with the mean annual rainfall as expected, but more sampled sites are needed in future research to draw conclusions. However, the occurrence of high *Culicoides* midge numbers at Hureb Süd coincided with the reports of higher AHS incidence proportion. Likewise, the sites with low *Culicoides* midge numbers (Neu Heusis and Isabis), reported few to no cases of AHS.

Neu Heusis was unusual, because the number of *Culicoides* midges collected there was much less than at areas of lower rainfall. With 420 mm/a, this is surprising, but their low numbers explain why AHS incidence proportion was low there.

Corona was unique in this study as it was found that despite its low mean annual rainfall location, the highest number of *Culicoides* midges was collected there, of which a large proportion was comprised of the proven AHS vector, *C. imicola*. Corona also had a large number of *E. z. hartmannae*, 20 donkeys and 50 horses, which meant there were both potential reservoirs and susceptible host animals comparable to other sites where AHS was prevalent. However AHS was not reported there. Horses were also never vaccinated on the farm, nor were any other preventative measures taken against AHS. Several questions arise from these observations: Why was there such an abnormally high number of *Culicoides* in an area with such low mean annual rainfall? Why, if all the needed components were present in the area, was AHS not observed in the horse population of Corona? Is it possible that AHSV is not circulated in the *E. z. hartmannae* and *Culicoides* midge populations in the area? Unfortunately no *E. z. hartmannae* were sampled for blood and tissue in the area and this is therefore a topic for further research. It is noted, however, that unlike the other farms, Corona had no cattle and horses were never kept stabled or in paddocks near the homestead. Further research is required to see if the high *Culicoides* midge numbers are perpetuated into summer. It must also be investigated whether or not anthropogenic modifications of the collection sites have an effect on the observed occurrence of *Culicoides* midges. Evaluations of the veld versus homestead *Culicoides* midge populations need to be investigated and are underway.

It appears that where in some instances the absence of AHS may be explained by the absence of *Culicoides* midges, in other cases it may be that host animals were not concentrated around sites which favoured *Culicoides* midge breeding, or both.

The vegetation density was reported to be sparse in the south-western Khomas Region, and provided the bare patches of soil are moist for some period, the area can potentially provide optimum breeding conditions for *Culicoides* species, such as the AHSV-vector *C. imicola* (Nevill, 1967; Braverman & Mumcuoglu, 2009). Throughout the area, despite the low mean annual rainfall, there are pools, earth dams and other possible moist 'islands' where *Culicoides* midge populations may be supported even during droughts. Also, given that on occasion the rainfall can increase three-fold in the area, this may imply that these few *Culicoides* midge survivors could become very numerous during favourable periods. The relatively high numbers of *Culicoides* midges collected in winter reveal that this may be the case. Further research will assess whether or not such an increase is actually observed during summer periods.

AHSV was found to occur in the *E. z. hartmannae* blood and tissues as evident by the presence of antibodies and viral RNA, indicating that *E. z. hartmannae* can become infected with AHSV. From this evidence that AHSV is circulating in endemic animals to the south-western Khomas Region, it is concluded that the outbreaks that have been observed could have originated from an endemic source and were therefore not necessarily due to a vaccine-induced disease. Insufficient coverage of horses in the area by vaccination may, however, explain why some vaccines did not effectively protect some animals.

No viable AHSV in zebra blood or tissues could be isolated in this study, and therefore *E. z. hartmannae* cannot be confirmed to be the principal in the area at this time. A larger sample is therefore required to answer this question, and a method devised to improve the quality of samples collected in the field. From a larger zebra sample, it must also be investigated whether or not there is a difference in the occurrence of the AHSV within different demographic groupings in the zebra population. Subclinical horses and donkeys in the area must also be screened for the presence of AHSV for their possible contributions to the maintenance and/or amplification of virus titre in the entire host population.

Vaccination applications were found to be too infrequently applied over the entire area to serve as effective protection for the horses being immunised. In the cases where vaccines failed to protect the horses, it may be due to the severity of the challenge from infections via *Culicoides* midge bites, where even a prepared immune system may be unable to react to the infection in time. Because of the presence of *E. z. hartmannae* across this entire area and (potentially more significantly) the presence of naturalised and unvaccinated horses, there are many potential hosts that can 'boost' or amplify virus levels, resulting in a large number of infected *Culicoides* midges. Different horse populations may have different susceptibility for AHS, which could affect the occurrence of AHS incidence proportion in one horse population versus another. This is a topic for further study.

## 4.2. Conclusion

The Hartmann's mountain zebra (*E. z. hartmannae*) is a cycling host in the area even if it forms only part of the entire reservoir pool, which may include adult *Culicoides* midges that are able to survive the winter.

On the basis of the winter *Culicoides* midge distribution and abundance, it is concluded that the *Culicoides* midge populations will be capable of sustaining virus transfer to hosts; either by maintaining the virus in the reservoir, by acting as reservoirs themselves or by transmitting the virus from the reservoir to susceptible hosts when their numbers increase during favourable periods.

From what is observed of the distribution and abundance of the vector of the AHSV (*Culicoides* species) and cycling host, *E. z. hartmannae*, it is concluded that AHS can be maintained in the south-western Khomas Region even at the lowest mean annual rainfall zones.