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Occurrence of *Culicoides* species (Diptera: Ceratopogonidae) in the Khomas region of Namibia during the winter months

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Summary

Although African horse sickness (AHS) is considered to be endemic in Namibia, limited data on its *Culicoides* midge vector (Diptera: Ceratopogonidae) are available. The principal study objective was to determine the presence, species composition and the richness and diversity of *Culicoides* adults during the colder and drier months in the Khomas region of central Namibia. Five sites were selected, ranging from relatively high to low altitudes with high to low annual rainfall. Onderstepoort suction UV-light traps were used for *Culicoides* species collection and were run during the winter from 6 July to 21 September 2009. A relatively high diversity of 25 species from 9 091 *Culicoides* individuals were collected in 34 collections. The abundance of the proven vector of AHS virus, *Culicoides imicola*, varied from 94% near Windhoek at a high altitude and relatively higher annual rainfall, to 12% at the site situated farthest south-west, with the lowest altitude and annual rainfall. This relatively high *Culicoides* midge abundance, coupled with the presence of a cycling host (zebra) in the area, imply that AHS virus may overwinter in the Khomas region of Namibia.

Keywords

African horse sickness, African horse sickness virus, *Culicoides*, *Culicoides imicola*, Khomas region, Midge, Namibia, Virus.

Specie *Culicoides* (Diptera: Ceratopogonidae): presenza nella regione del Khomas in Namibia durante i mesi invernali

Riassunto

Sebbene la peste equina (AHS) sia considerata endemica in Namibia, sono disponibili dei dati seppur limitati riguardanti il suo vettore chiamato Culicoides *midge (Diptera: Ceratopogonidae). L'obiettivo principale dello studio è stato quello di determinare la presenza, la composizione delle specie, nonché la ricchezza e la diversità dei* Culicoides *adulti durante i mesi più freddi ed asciutti nella regione del Khomas situata nella zona centrale della Namibia. Sono stati selezionati cinque siti e sono stati selezionati tenendo conto sia dell'alta e bassa quota sia delle copiose o scarse precipitazioni piovose annuali. Sono state utilizzate le trappole a risucchio Onderstepoort con lampade UV per la raccolta di specie di* Culicoides. *Questo lavoro è stato portato avanti, durante l'inverno locale, dal 6 luglio al 21 settembre 2009. E' stata selezionata una diversità piuttosto alta di 25 specie, provenienti da 9091* Culicoides *in 34 tipi di raccolta. La presenza abbondante del comprovato vettore AHS (AHSV),* Culicoides imicola, *variava dal 94% nei pressi di Windhoek ad alta quota con elevate precipitazioni piovose annuali, sino al 12%*

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presso il sito che si trova nel punto più lontano a sud-ovest, a quota più bassa e con frequenti precipitazioni piovose. Tutto quanto sopra premesso e tenendo anche conto dell'alta concentrazione del Culicoides Ceratopogonidae *e la presenza di un ospite portatore (zebra) nella zona, si può affermare che il vettore AHSV sverna nella regione del Khomas della Namibia.*

Parole chiave

Culicoides, *Culicoides Ceratopogonidae*, *Culicoides imicola*, Namibia, Peste equina, Regione del Komas, Virus, Virus della Peste equina.

Introduction

Certain species of biting midges of the genus *Culicoides* (Diptera: Ceratopogonidae) are considered to be vectors of several serious orbiviral (*Reoviridae*) diseases that are classified as 'notifiable' by the World Organisation for Animal Health (*Office International des Épizooties*: OIE) (see Meiswinkel *et al*. [10] for a review). Of these diseases, African horse sickness (AHS) is endemic to sub-Saharan Africa (3), but occasionally outbreaks occur in other warm climes, such as India, Morocco, Pakistan, Portugal and Spain (6, 9, 11).

In southern Africa, *Culicoides* species become abundant in the second half of the summer, in warm, moist conditions (10). They are most common in low-lying areas, such as coastal regions, marshes, riverine environments and valleys (3). Eggs and larvae are sensitive to desiccation (13), while temperature affects larval development and adult survivorship (1, 8). In the proximity of livestock, *Culicoides imicola* can become superabundant under the afore-mentioned favourable conditions, in which case millions may be caught with a light trap in a single night (10). In frost-free areas of South Africa, this species can be active and is able to breed all year round (10, 18).

AHS is considered endemic to Namibia (7). Outbreaks of the disease in Spain have been attributed to 10 zebras (*Equus burchelli*) imported from Namibia (5, 9). It was also found that 50% of donkeys assayed in the Windhoek area demonstrated AHS antibodies in their blood (19). Four of the nine known AHS virus (AHSV) serotypes (AHSV 1, 2, 4 and 9) were recently isolated from horses around the Windhoek area (15).

Although there are no official records of the disease in the south-western Khomas region, farmers have reported several horse casualties in the area, which was rather unexpected, as the area is particularly dry – conditions thought unfavourable for *Culicoides* midges. A total of 95% of Namibia receives less than 500 mm annually and the mean annual rainfall is estimated at 270 mm (17). Although Enderlein described the first sub-Saharan *Culicoides* species in Namibia in 1908, knowledge of *Culicoides* midges in Namibia is lacking, especially during the dry, winter months. Investigation into their activity over this period may also contribute to the understanding of their overwintering strategies. If adult *Culicoides* midges can survive the winter, so too can the AHSV by inhabiting these infected adult midges. This has implications for possible outbreaks of AHS in favourable conditions in Namibia.

This study therefore aims to determine whether adult *Culicoides* midges can survive the colder, drier months of the study area. In addition, this survey provides an indication to species composition, species richness and diversity in central Namibia.

Materials and methods

Study area

The study area shown in Figure 1, (22°24.063′S, 17°01.791′E; 23°32.617′S, 15°53.870′E) is situated in undulating terrain (12) where AHS was reported by local farmers. Aside from Windhoek, a city with a population of 233 529 people (16), the human presence of the rural south-western Khomas region is sparsely distributed (12) and the most common land uses include cattle farming, horse breeding and private reserves/guest farms (E. Becker, personal observation). The arid conditions restrict stocking rates to less than one head of cattle per square kilometre (12). Wild animals, such as Hartmann's mountain zebra (*Equus zebra hartmannae*), kudu (*Tragelaphus strepsiceros*) and oryx (*Oryx gazelle*), occur

naturally in the area. No large-scale crop irrigation is practised in the study area.

Collections were made at five sites (Fig. 1). Except the collections made at the Avis horse stables, all locations were in rural settings. The altitudes of the locations ranged from 900 m to 1 800 m above sea level. The area receives summer rainfall which ranges from an annual average of 420 mm to 120 mm (landowners' communication). Winters can be relatively mild with only 0-10 days of frost (12).

Farmers measure and keep records of rainfall on their farms. After the *Culicoides* midge collection period, they were contacted by telephone to report on rainfall events and dates.

Collection sites

Avis

The Avis horse stables (22°34.200′S; 17°5.016′E) are situated near the Avis Dam in Windhoek, at an altitude of 1 650 m above sea level. The dam is solely used for recreational purposes; thus there were no irrigation activities in the area, except watered gardens from residences. Horses were kept at high densities at the stables, particularly at night. The stalls had no doors and horses can stand outside at night in small encampments in front of their stalls The trap was affixed to an outbuilding within 3 m of the nearest stalls and directly adjacent to several paddocks. Water spillage on vegetation-free soil was observed. According to the Namibia Meteorological Services, the average annual rainfall is estimated at 360 mm.

Neu Heusis

Neu Heusis homestead (22° 36.660′S, 16° 42.646′E) is situated on the watershed at an altitude of 1 739 m above sea level. The stables often contained five to six young horses for the greater part of the day, which remained stabled overnight. About ten to fifteen horses roamed free in surrounding paddocks. The trap was affixed to the stable within 5 m of the stable door and about 10 m from the paddocks.

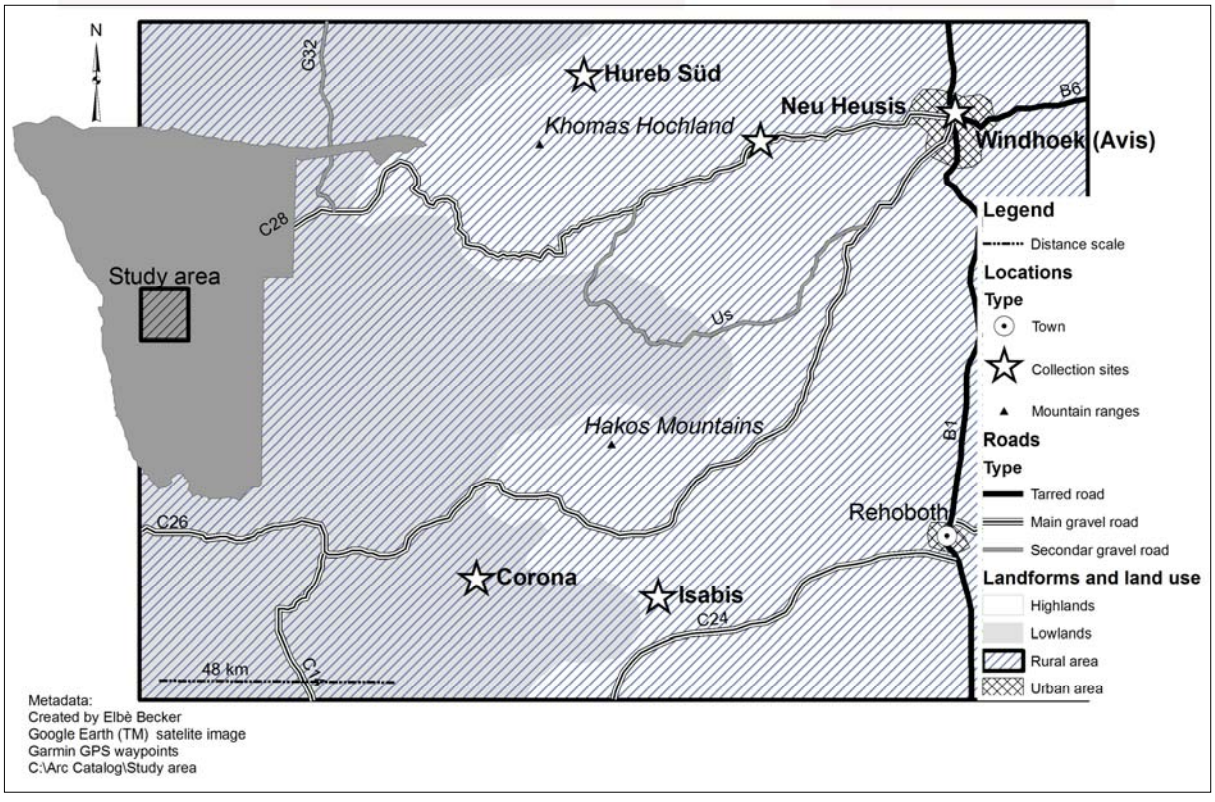

Figure 1
Study area showing *Culicoides* midge collection sites: Avis, Neu Heusis, Hur eb Süd, Isabis and Corona in the south-western Khomas region of Namibia, from July to September 2009

Very little water spillage was visible in the surroundings. Surface waters were present in nearby water troughs only – with no signs of leakage. The garden, some 50 m away, consisted of xerophytic plants and did not appear to be watered regularly. The estimated annual rainfall at Neu Heusis is 420 mm (landowner's communication).

Hureb Süd

Hureb Süd homestead (22°29.394′S; 16°22.172′E) is at an altitude of 1 216 m above sea level. The trap was installed in the homestead garden. Several vegetation-free paddocks, some 20 m from the trap, occasionally held prized horses. There were two stables where horses were seldom kept 15 m from the trap, with an exception made for mares with their foals and/or sick horses. Horses were bred on the farm and therefore totalled over 50 animals which had free roam of the farm. However, their movements were restricted to the vicinity of watering points, including one at the homestead area some 100 m from the trap. No water spillage around watering troughs was observed. There were, however, outdoor taps used for rinsing where pools occasionally formed. The garden was not moist, nor the plant growth lush, except for a ground-creeper which covered a large portion of the garden almost directly below the trap. The estimated annual rainfall at Hureb Süd is 250 mm (landowner's communication).

Isabis

Isabis homestead (23°25.394′S, 16°30.894′E) is situated on the Namibian plateau, at an altitude of 1 639 m above sea level. The trap was installed at open windows of stables containing three stalls. It was in close proximity to paddocks (about 6 m) in which cattle were occasionally kept. Several horses were kept in surrounding camps more than 100 m from the trap. Semi-feral horses roamed the farm freely. No water spillage was observed in the surrounding area during the study period. The garden, some 50 m away, was shady and lush – it appeared to be watered regularly. Open waters were found in water troughs and a nearby reservoir, but no seepage onto the soil was observed from these sources. The estimated annual rainfall at Isabis is 220 mm (landowner's communication).

Corona

Corona homestead (23°23.444′ S, 16°09.600′E) is situated at an altitude of 1 185 m above sea level at the foot of the escarpment. There were no stables, but the trap was affixed to the side of a building which faced a large wildlife enclosure with a water trough (some 20 m from the trap), which horses frequented. Horses were the only domesticated animals kept on the farm. About 20 to 25 horses roamed freely on the farm. Water seepage from an irrigation pipe into garden refuse was observed some 5 m from the trap. The nearby garden (15 m from the trap) was lush and watered regularly. The estimated annual rainfall at Corona is 120 mm (landowner's communication).

A telephonic survey revealed that no rainfall was reported by farm owners during the test period.

Collection methods

Collections were made during the winter from 6 July to 21 September 2009 using the Onderstepoort 220V suction UV-light trap (21). The traps were emptied once a week by the farmers on whose property the traps were installed. Most species of biting midges are active from sunset to sunrise (10); the traps were therefore only operated between these hours and regulated by a Toptronic® programmable time switch (model TDDT7).

Collections were made in 30% ethylene glycol solution to preserve the samples until the week's end. Samples were transferred to 70% ethanol solution, wherein the specimens were preserved (4). At the Agricultural Research Council (ARC)-Onderstepoort Veterinary Institute (OVI), the number of *Culicoides* midges in each sample was determined and identified to species level. The midges were identified morphologically using the wing picture atlas of Afrotropical *Culicoides* (R. Meiswinkel 1994, unpublished data).

The number of *Culicoides* species and abundance per species at each site was used to determine species richness (Margalef index), which takes sample size into account. Species

diversity was calculated using the Shannon index, which describes the evenness in distribution of abundance of species. These indices were calculated by use of Primer 5 software for Windows, version 5.2.9 (Copyright 2002 Primer-E Ltd).

Results

From 6 July to 21 September, 34 light trap collections were made at the five trap sites and 9 091 *Culicoides* individuals belonging to 25 species were collected. The mean number of *Culicoides* species collected weekly at each site is given in Table I. The relative species composition and abundance at each collection site for the sampling period is given in Table II. The total number of species, individuals, species richness and Shannon diversity indices for each site are given in Table III.

Table I
Mean weekly *Culicoides* species collections made in UV-light traps at five sites in the Khomas region of Namibia from July to September 2009
(standard deviation in brackets)

Culicoides species	Location				
	Avis	Neu Heusis	Hureb Süd	Isabis	Corona
C. cornutus			0.2 (0.4)		
C. expectator	0.3 (0.8)		20.7 (17.4)		0.1 (0.4)
C. herero	0.5 (0.8)	0.2 (0.3)	24.8 (50.8)	1.25 (1.5)	54 (134.3)
C. imicola	221 (156,4)	12.5 (7.5)	82.8 (76.7)	26 (4.4)	73.6 (9.9)
C. leucostictus	1.3 (1.9)	2.1 (1.3)	10.8 (16.6)	1.5 (2.4)	7.1 (9.2)
C. pycnostictus	2.2 (1.7)	11 (6.3)	3.5 (4.4)	10.8 (9.9)	54.4 (43.1)
C. ravus	1.3 (2.2.)	23.8 (3.5)	184.2 (323.1)	44.3 (66.5)	264.6 (480.3)
C. subschultzei	7.7 (8.4)	15.1 (36.6)	70.2 (128.3)	3.3 (5.9)	56.9 (102.0)
C. tropicalis	0.3 (0.8)	0.1 (0.3)	2.3 (3.3)	2.8 (3.8)	5.25 (10.9)
C. pretoriensis			0.2 (0.4)		0.3 (0.7)
C. schultzei	0.3 (0.5)		1.7 (2.9)	0.3 (0.5)	0.1 (0.4)
C. tuttifrutti				4.5 (8.3)	1.0 (2.4
C. macintoshi		0.1 (0.3)	5.3 (8.6)	0.3 (0.5)	1.0 (1.8)
C. #90		0.1 (0.3)			
C. kanagai		0.2 (0.4)			
C. bedfordi			0.5 (1.2)		0.3 (0.5)
C. #94		0.2 (0.6)			1.0 (1.8)
C. #89					0.3 (0.7)
C. brucei	0.2 (0.4)	0.1 (0.3)			1.9 (2.8)
C. magnus					0.3 (0.7)
C. olyslageri					0.1 (0.4)
C. nivosus	0.2 (0.4)	0.1 (0.3)	2.7 (5.2)		
C. remerki			0.5 (1.2)		
Accraensis group		0.1 (2.6)			0.3 (0.7)
C. similis					0.3 (0.7)
Total collection/week	235	66	410	95	597

Table II
Relative *Culicoides* species composition from collections made in UV-light traps at five sampled sites in the Khomas region of Namibia from July to September 2009

Culicoides species	Collection site									
	Avis		Neu Heusis		Hureb Süd		Isabis		Corona	
	No. of collections									
	6		10		6		4		8	
	% of site total	% of grand total	% of site total	% of grand total	% of site total	% of grand total	% of site total	% of grand total	% of site total	% of grand total
C. cornutus					0.4	*				
C. expectator	0.1	*			5.0	1.6			*	*
C. herero	0.2	*	0.3	*	6.1	1.9	1.3	0.1	9.0	4.1
C. imicola	93.9	16.6	19.0	0.9	20.2	6.2	27.4	2.0	12.3	5.5
C. leucostictus	0.6	0.1	3.2	0.2	2.6	0.8	1.6	0.1	1.2	0.5
C. pycnostictus	0.9	0.2	16.7	0.8	0.9	0.3	11.3	0.8	9.1	4.1
C. ravus	0.6	0.1	36.2	1.8	44.9	13.9	46.7	3.3	44.3	19.9
C. subschultzei	3.3	0.6	23.0	1.1	17.1	5.3	3.4	0.2	9.5	4.3
C. tropicalis	0.1	*	0.2	*	0.6	0.2	2.9	0.2	0.9	0.4
C. pretoriensis					*	*			*	*
C. schultzei	0.1	*			0.4	0.1	0.3	*	*	*
C. tuttifrutti							4.7	0.3	0.2	0.1
C. macintoshi			0.2	*	1.3	0.4	0.3	*	0.2	0.1
C. #90			0.2	*						
C. kanagai			0.3	*						
C. bedfordi					0.1	0.0			*	*
C. #94			0.3	*					0.2	0.1
C. #89									*	*
C. brucei	0.1	*	0.2	*					0.3	0.1
C. magnus									*	*
C. olyslageri									*	*
C. nivosus	0.1	*	0.2	*	0.6	0.2				
C. remerki					0.1	*				
Accraensis group			0.2	*					*	*
C. similis									*	*
Percentage of grand total catch	18.7		5.2		32.6		7.1		36.4	

* *Culicoides* species comprising less than 0.1% of the site and/or total collection

Table III
Total number of *Culicoides* species, individuals, richness and diversity indices from UV-light collections made at five sampled sites in the Khomas region of Namibia from July to September 2009

Site	Total species (S)	Total individuals (N)	Margalef species richness index (*d*)	Shannon's diversity index (H′ [$\log_e$])
Avis	11	1412	1.379	0.3236
Neu Heusis	14	657	2.004	1.543
Hureb Süd	15	2 462	1.793	1.605
Isabis	10	379	1.516	1.475
Corona	20	4 181	2.279	1.521

Collection sites

Avis

At Avis, 1 412 *Culicoides* midges comprising 11 species were collected over six weeks (Table III), with about 235 *Culicoides* midges per week (Table I). Of the 11 species collected, *C. imicola* dominated he collection at 93.9% and accounted for 16.6% of all *Culicoides* species collected during this survey (Table II). The second largest representative was *C. subschultzei* at 3.3% of the site collection and 0.6% of the total number of *Culicoides* species collected (Table II). Of the five sites sampled, Avis had the lowest diversity index of H′ = 0.3236 and the lowest species richness index at *d* = 1.379 (Table III). There were no zero values during the test period.

Neu Heusis

The lowest number of midges was collected at Neu Heusis (Table I) and it accounted for only 5.2% of the total number of midges collected in this survey (Table II). A total of 657 *Culicoides* midges were collected over a period of ten weeks (Table III), that is about 66 *Culicoides* individuals per week (Table I). The dominant species was *C. ravus* which comprised 36.2% of the collection. *C. subschultzei* had the second largest representation at 23.0% of the site catch and 1.1% of the total number of *Culicoides* collected (Table II). Despite the low numbers collected, 14 species were present in the collections, resulting in a diversity index of H′ = 1.543 and a richness index of *d* = 2.004 (Table III). This was the only site where *Culicoides* midges were absent in the trap for a week or longer. During the first five weeks (6 July 2009 to 3 August 2009), no *Culicoides* were collected in the light traps.

Hureb Süd

Hureb Süd was the second most productive site and 2 462 *Culicoides* were collected over a period of six weeks (Table III), that is about 410 *Culicoides* midges per week (Table I). Of the 15 species collected, the dominant species was *C. ravus* representing 44.9% of the site collection and accounting for 13.9% of the total number of *Culicoides* midges collected in this survey (Table II). It was seconded by *C. imicola* comprising 20.2% of the site collection and 6.2% of the total *Culicoides* midge collection (Table II). The diversity index for Hureb Süd was H′ = 1.605 and the richness index *d* = 1.795 (Table III).

Isabis

At Isabis, a total of 379 *Culicoides* were collected over a period of four weeks (Table III), about 95 *Culicoides* individuals per week (Table I). Ten species were represented at Isabis (Table III). The dominant species was *C. ravus* representing 46.7% of the site collection and 3.3% of the total number of *Culicoides* midges collected. It was seconded by *C. imicola* at 27.4% and 2.0% of the total *Culicoides* collection (Table II). Isabis had a diversity index of H′ = 1.475 and a species richness index of *d* = 1.516 (Table III).

Corona

The biggest collections were made at Corona where 4 181 *Culicoides* midges were collected over a period of seven weeks (Table III), an average of 597 *Culicoides* midges per week (Table I). Twenty species were represented at Corona (Table III), of which the dominant

species was *C. ravus*, representing 44.3% of the *Culicoides* midges collected at this site and 19.9% of the total number of *Culicoides* midges collected in the survey. It was seconded by *C. imicola* at 12.3% of the site collection and 5.5% of the total *Culicoides* midge collection (Table II). The diversity index was H′ = 1.521 and the species richness index was *d* = 2.279 – the highest of all sites sampled (Table III).

Discussion and conclusion

The relatively high diversity of *Culicoides* species, as well as the relatively high abundance during the dry winter months in the Khomas region was unexpected.

With the exception of Avis, all sites revealed that the dominant species was *C. ravus* (Table II), followed either by *C. imicola* or *C. subschultzei*. *C. imicola*, considered a proven vector of AHSV, was most abundant at Avis comprising 16.6% of the total number of *Culicoides* species collected, followed by Hureb Süd (6.2%) and Corona (5.5%). These two locations of high *C. imicola* abundance are separated by two regions of lower *C. imicola* representation at Isabis (2.0%) and Neu Heusis (0.9%). *C. imicola* represented 93.9% of the midges collected at Avis, 19.0% at Neu Heusis, 20.2% at Hureb Süd, 27.4% at Isabis and 14.1% at Corona.

Of the 25 *Culicoides* species collected, seven (*C. imicola*, *C. ravus*, *C. herero*, *C. leucostictus*, *C. pycnostictus*, *C. subschultzei* and *C. tropicalis*) were collected at all five sites. A further seven species, *Culicoides #90* (the numbering system used is that of R. Meiswinkel and refers to yet undescribed *Culicoides* species), *C. kanagai*, *Culicoides #89*, *C. magnus*, *C. olyslageri*, *C. remerki* and *C. similis* had a more limited or patchy distribution (Table II). Although *C. expectator*, *C. herero*, *C. tuttifrutti*, *C. macintoshi*, *C. nivosus* and *C. tropicalis* were found to be widespread, the numbers collected per location were relatively low. Other species that were collected at more than one location, but had a low percentage representation were as follows: *C. cornutus*, *C. pretoriensis*, *C. schultzei*, *C. bedfordi*, *Culicoides #94*, *C. brucei*, *C. nivosus* and a *Culicoides* species belonging to the Accraensis group (Table II).

At the Avis stables, diversity was low (Table III) and *C. imicola* was strongly dominant with no clear secondary dominant species (Table II). The environment at Avis is also different from the other sites sampled, as it is located in an urban area. The low diversity of the site (H′ = 0.3236), coupled with the strong *C. imicola* dominance at the 'expense' of representation by other species (the species richness index was only *d* = 1.379), may indicate a disturbance in the natural ecology of *Culicoides* species due to anthropogenic activity. Further research is underway to address this issue. The other locations (Neu Heusis, Hureb Süd, Isabis and Corona) had species diversity and richness indices that were much closer in value: H′ = 1.543; 1.605; 1.475; 1.521 and *d* = 2.004; 1.759; 1.516; 2.279, respectively. Avis had the highest host densities, yet the lowest *Culicoides* species diversity – supporting the findings by Meiswinkel *et al*. (10) that host density may play a major role in the abundance of species like *C. imicola*.

Yet, it was interesting that Corona had such a high *Culicoides* midge count despite being the site in the lowest rainfall zone and the lowest average host density in the vicinity of the trap. In addition, there were no cattle kept on the farm. Corona recorded the highest species richness even when taking the larger sample size obtained from Corona into consideration, with a Margalef index of *d* = 2.279. The Hureb Süd trap also had a surprisingly high *Culicoides* midge count with a comparatively low host density at the trap surroundings. Its richness index was slightly lower at *d* = 1.795, but it was also the most diverse site at H′ = 1.605. This seems to suggest that large mammal host density could have a greater effect on dominance and species diversity than on the total number of *Culicoides* midges present in an area. An exception to this assumption is presented by the Neu Heusis results. Of the rural sites, it had the highest mammal host density around the trap, yet the lowest weekly total *Culicoides* midge collection was made there. Both species richness and diversity were

comparatively high at $d = 2.004$ and $H' = 1.543$. This suggests the possible combined effect of breeding site availability and host densities – with breeding site availability acting as a limiting factor on host availability.

Those areas of higher altitude and more undulating topography, Neu Heusis and Isabis, showed lower total *Culicoides* midge collections than the lower altitude locations, Hureb Süd and Corona (Table III). It is possible that the altitude and topography affects availability of breeding sites and the distribution of wild animals which may be potential hosts. Hartmann's mountain zebra, for instance, prefers the more rugged terrains (2) which are characteristic of Hureb Süd and Corona.

Culicoides midge activity did not cease over the dry season in the south-western Khomas region and its species richness is high compared with higher rainfall regions, such as the Stellenbosch area (20) where 15 species were collected in 16 sites over a period of one year, compared to the 25 species collected at only five sites over a period of less than three months in the current study.

At least five of the species collected in this study, namely: *C. imicola*, *C. pycnostictus*, *C. subschultzei*, *C. schultzei* and *C. magnus* are considered to be high potential orbivirus vectors, based on their host preference and virus isolation from field collected samples from collections made across South Africa (14).

Although water usage and/or spillage at all locations were by no means profuse, the available soil moisture is expected to be more than it would have been had the sites not been modified by human activity. The 'islands' of higher soil moisture is expected to influence *Culicoides* midge numbers. Research is underway to determine whether significant differences in *Culicoides* midge numbers exist between homestead and veld locations.

The implication of these findings is that the abundance of *Culicoides* species during periods of the above normal rainfall summer months is expected to be high. This relatively high *Culicoides* midge abundance, coupled with the presence of a cycling host (zebra) in the area, imply that AHSV may overwinter in the area. The high species diversity found and the presence of a number of species of which little is known regarding their biology and potential as vectors for arboviruses, will add to the complexity of the epidemiology of AHSV in Namibia.

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