The effect of anthropogenic activity on the occurrence of Culicoides *species in the South-Western Khomas Region, Namibia*

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Anthropogenic activity,
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Culicoides imicola,
Khomas region,
Midge,
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Natural occurrence.

Summary
Certain species of midges in the genus *Culicoides* (Diptera: *Ceratopogonidae*) are vectors of several serious orbiviral (*Reoviridae*) diseases, one of which, African horse sickness (AHS), was reported in the South-Western area of Khomas Region, Namibia, where it had been believed to be absent. *Culicoides imicola*, AHS principal vector, was collected in several farms in the area during the winter of 2009. The objective of this study was to determine whether *Culicoides* midges, especially *C. imicola*, were favoured at anthropogenic impacted/homestead sites in the arid Khomas Region, where they were not expected to occur under natural, veld conditions. The natural 'background' *Culicoides* communities where determined from collections made at veld sites, which were then compared to corresponding collections made at homestead sites. Altogether, 10,178 *Culicoides* midges were collected at homesteads and were then compared to 1,733 individuals collected at veld sites. *Culicoides* midge numbers were likely boosted in anthropogenic impacted areas/homesteads. This was also the case for the *Culicoides* species that are vector of AHS. This study indicated the significance of human settlement in the Khomas Region in terms of *Culicoides* midge abundance and distribution and showed the implications that this may have on the transmission of *Culicoides*-vectored diseases.

Effetto dell'attività antropogenica sulla presenza di Culicoides *nell'area Sud-Occidentale della regione di Khomas, Namibia*

Parole chiave
Attività antropogenica,
Culicoides,
Culicoides imicola,
Moscerino,
Namibia,
Regione di Khomas,
Virus della peste equina.

Riassunto
Alcune specie appartenenti al genere *Culicoides* (Diptera: *Ceratopogonidae*) sono vettori di numerose e gravi patologie da *Orbivirus* (*Reoviridae*) tra cui la peste equina, riscontrata nell'area Sud-Occidentale della regione di Khomas in Namibia, dove invece si riteneva che fosse assente. Durante l'inverno del 2009, esemplari di *Culicoides imicola*, principale vettore del virus della peste equina, sono stati rilevati in diverse aziende agricole della regione. Lo studio ha avuto l'obiettivo di verificare se le attività antropogeniche presenti nell'area favorissero la presenza di *Culicoides*, in particolare di *C. imicola*, che generalmente sono raramente rilevati nelle aree non coltivate. I dati relativi alla cattura di *Culicoides* in aree con attività antropogenica, come fattorie, sono stati comparati con quelli relativi a zone incolte. Complessivamente, in prossimità delle aziende agricole sono stati catturati 10.178 esemplari di *Culicoides* che sono stati successivamente comparati con 1.733 esemplari prelevati da aree incolte. L'elevato numero di *Culicoides* è stato verosimilmente determinato dalla vicinanza alle aziende agricole, dove peraltro sono state rilevate le specie di *Culicoides* vettori della peste equina. Lo studio mostra gli effetti degli insediamenti umani sulla presenza e distribuzione di esemplari di *Culicoides* nell'area Sud-Occidentale della regione di Khomas, evidenziandone le possibili implicazioni nella trasmissione delle patologie di cui i *Culicoides* sono vettori.

Introduction

Certain species of blood feeding midges in the genus *Culicoides* (Diptera: *Ceratopogonidae*) are considered the biological vectors of viruses, protozoa and filarial nematodes, which affect birds, humans and other animals (14, 16). Among the orbiviruses (*Reoviridae*) transmitted by certain *Culicoides* species, those causing bluetongue (BT), African horse sickness (AHS), equine encephalosis (EE) and epizootic haemorrhagic disease (EHD) are of major veterinary significance (14, 16). At least 3 of these diseases, BT, AHS and EHD are classified as 'notifiable' by the World Organisation for Animal Health (*Office International des Épizooties*: OIE) (14, 16).

Due to the arid climate of the South-Western part of the Khomas Region in Namibia, *Culicoides* midge abundance and the associated risk for orbiviral transmission was expected to be negligibly low (3). Consequently there was no economic or veterinary incentive to collect *Culicoides* midges in the region. However, reported outbreaks of AHS vectored by *Culicoides* (*Avaritia*) *imicola* and *Culicoides* (*Avaritia*) *bolitinos* (6, 8, 16, 19) in the area changed this view (3). The risk of orbiviral transmission was furthermore highlighted by the fact that *Equus zebra hartmannae* (Hartmann's mountain zebra), a suspected reservoir for AHSV (5, 10), lives in the area and tested positive for circulating antibodies against the disease (3).

Not much is known about the occurrence of *Culicoides* midges in Namibia as a whole and this study will also provide some indication of the occurrence of *Culicoides* midges in previously undocumented geographical locations. A recent light trap survey conducted from 2009-2010 yielded a surprisingly large number of *Culicoides* in the arid South-Western part of the Khomas Region (4). However, because of the dependency of the Onderstepoort suction UV-light traps (24) on 220V electricity, collection sites were restricted to farm homesteads sites where electricity was available. These homestead sites were unavoidably situated in habitats that were also impacted by anthropogenic activity. It should be noted that, due to the low human population, the greatest part of the Khomas Region is mostly unaffected by anthropogenic activity (17). These homesteads sites are therefore atypical of the South-Western part of the Khomas Region.

Culicoides midges depend on moisture (7, 14) both for breeding substrates and for surviving the high temperatures (1, 12). The homestead sites were expected to have more moisture due to garden irrigation (water is supplied via boreholes) and spillage (4), which is uncharacteristic of the predominantly natural veld and undeveloped farmland of the area. The anthropogenic impacted habitats at homesteads showed also a more consistent presence of livestock, kept at relatively higher densities than the one typically found across the region. Livestock animals serve as vertebrate hosts for many *Culicoides* species (22) - e.g. *C. imicola* and *C. bolitinos* (19) - and may attract and support them in relatively large numbers.

Worldwide, most studies have focussed on the occurrence of *Culicoides* midges found around livestock at homesteads (20), while very few investigations into the occurrence of *Culicoides* midges in areas distant from anthropogenic habitats have been conducted. It is expected that the occurrence of *Culicoides* midges in habitats impacted by anthropogenic activity may differ significantly from non-anthropogenic impacted habitats, the knowledge of which may contribute to the understanding of many ecological relationships involving *Culicoides* midges, such as the transmission of vector-borne diseases among wild, indigenous hosts (20).

The objectives of this study were to investigate whether these anthropogenic impacted habitats may have influenced the abundance of *Culicoides* midges and to assess whether collections made at these habitats would be representative of the region in general. To achieve these objectives, the natural veld or 'background' *Culicoides* populations collected with light traps at veld sites, away from homesteads, were compared to the *Culicoides* populations collected at farm homesteads.

Materials and methods

Study area

The South-Western part of the Khomas Region has a semi-desert to desert climate (17), with summer rainfall ranging from 420 mm/a in the North-East to 120 mm/a in the South-West (3). Winters are mild with, on average, 0 to 10 days of frost per year (17). The landscape is undulating, but can be differentiated into the highland plateau, steep escarpment and desert pediment. The escarpment is drained exorheically by the Kuiseb, Oanob and Swakop River basins, although rainfall is mostly too low for the ephemeral flows of the rivers to reach the sea. Several smaller ephemeral rivers end blindly inland (endorheic rivers) in dunes or inland pans (11). However, the river beds are dotted with permanent to semi-permanent rock pools. These pools, as well as earth dams, reservoirs and animal watering troughs, are scattered sparsely throughout the landscape and are the only surface water in the area. The vegetation varies from highland savannah on the plateau in the East to a shrub-dominated transition zone on the escarpment and sparser pre-Namib vegetation on the pediment in the West [Giess, 1971 as quoted by Joubert (11)]. Riparian

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vegetation in ephemeral river beds (11) differs distinctly from the surrounding areas and shows tall trees, which often form a closed canopy.

Agriculture is the main land use of the study area (3, 17). Due to the low annual rainfall, animal husbandry, rather than crop cultivation, is preferred, while irrigation is limited to small homestead gardens. Cattle-ranching is the most common agricultural practice. Nonetheless, stocking rates are low, at an average of one head of cattle per 10 km^2 (3). Wild animals such as *Equus zebra hartmannae*, *Tragelaphus strepsiceros* (kudu) and *Oryx gazelle* (oryx) roam freely in the area.

Collection Sites

The area was surveyed between February the 27th and October the 6th 2010 for *Culicoides* midges. Light trap collections were made at homestead and veld sites on 4 farms (Neu Heusis, Hureb Süd, Isabis/ Alberta and Corona).

Neu Heusis

Homestead site (22°36.660´ S, 16°42.646´ E)

Neu Heusis homestead (1,739 m above sea-level) (Figure 1) is situated on the watershed of the Kuiseb River, where the relief is gentle and the landscape even. The UV-light trap was fixed to the outer wall of a horse stable housing young horses on occasion. A variable number of horses (10-15), some 10 m away, were present in very large encampments surrounding the homestead area. No water leakage was observed from water troughs. The garden vegetation was generally sparse and not irrigated regularly. *Culicoides* midges were collected on February the 27th, March the 27th and 28th, May the 22nd and 24th 2010.

Veld site (22°37.773´ S, 16°45.107´ E)

The corresponding veld site (1,707 m) was located 5 km away from the Neu Heusis homestead site (Figure 1). The trap was situated on the side of a hill overlooking an ephemeral stream. A tap leaking water, which formed a large mud pool, was observed about 1.5 km from the trap. The only other moist substrate observed was cow dung. None of the 5 ephemeral pools held water during the study. *Culicoides* midges were collected during the same nights as at the homestead site.

Hureb Süd

Homestead site (22°29.394´ S, 16°22.172´ E)

Hureb Süd homestead (1,216 m above sea-level) is situated on the escarpment of the Khomas Hochland

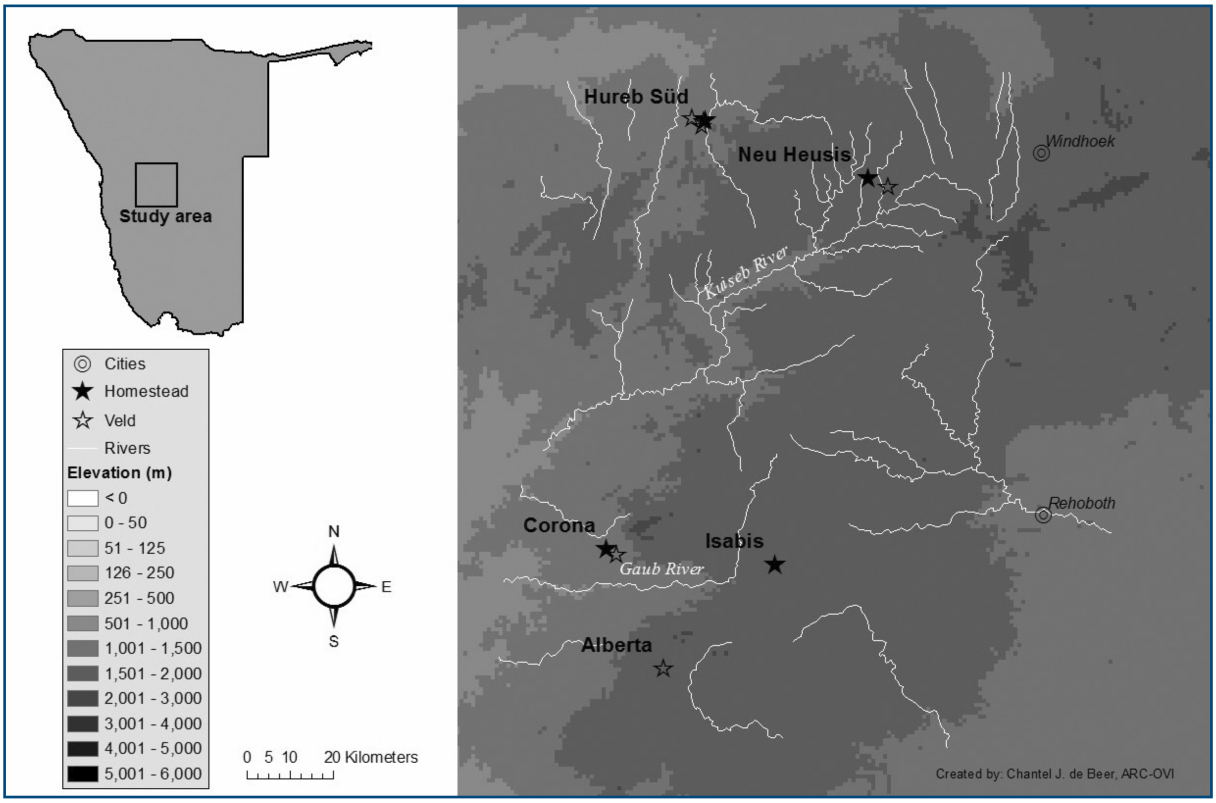

Figure 1. *The study area with surveyed sites for* Culicoides *midge collections from February the 27th to October the 6th 2010; Neu Heusis, Hureb Süd, Isabis and Corona in the South-Western Khomas region, Namibia.*

(Figure 1). The homestead was located within the bluff line of an ephemeral river abounded by hills and spurs. The trap was installed on a building in the garden. Some water spillage was observed from outdoor taps in the yard, which was largely vegetation-free. Horses were occasionally kept in door-less stables and paddocks some 15 and 20 m from the trap. Although the horse herd had free-roam of the farm, they were often concentrated at watering points, situated some 100 m from the trap. The nearby river had not flowed for more than a year. *Culicoides* midges were collected on March the 7th, May the 19th and 21st, June the 30th and October the 6th 2010.

Veld site 1 (22°37.773´ S, 16°45.107´ E)

Four collections were made at this Hureb Süd veld site (1,425 m above sea-level) located 3 km from the homestead site. The trap was situated on the side of a mountain. There were several zebra roll-holes in the area; some of which may fill with water after rain to form pools that can last for up to a week. Neither livestock nor their dung was found in about a 3 km radius of the trap. A visual survey of the area of about 2 km radius revealed no further moist habitats. Any dung that was found was very dry. Collections were made shortly after the wet season on March the 7th and from the 19th to 21st of May 2010.

Veld site 2 (22° 29.506' S, 16° 21.824' E)

This veld site was located in the same ephemeral river bed which runs past the homestead. There were many tall trees in the ephemeral river, unlike the rest of the area. Moist substrates were present in tree-hollows and clumps of rotting vegetation material. Dry ephemeral pools were also present, which may hold water after rains or after the river had been in flow. A high concentration of fresh dung was found within the river bed. *Culicoides* midges were collected on June the 30th 2010.

Veld site 3 (22° 30.052' S, 16° 21.655' E)

The site was very similar to the second Hureb Süd veld site in terms of habitat type. It is situated in a river bed with many tree-hollows, caves and sheltered areas. *Culicoides* midges were collected on October the 6th 2010.

Isabis

Homestead site (23°25.394´ S, 16°30.894´ E)

Isabis homestead (1,639 m above sea-level) is situated on the plateau and the average slope is gentle. The trap was installed in stables close to cattle paddocks. Several horses were kept in large encampments around the homestead. The garden was about 50 m away from the trap and was regularly watered. The surrounds of the homestead were covered by a dense growth of native grass species. *Culicoides* midges were collected from May the 14th to May the 16th 2010, after the wet season.

Veld site (23°38.502´ S, 16°16.728´ E)

The corresponding veld site collection of the Isabis homestead collections had been conducted on the farm Alberta 34 km away. Alberta is situated 1,783 m above sea-level, on the plateau and located within the same rainfall zone as the Isabis homestead (Figure 1). The trap was installed in an ephemeral river bed, which is in a wide valley but for its Southern side where it is bordered by a hill. There were very few trees and herbaceous shrubs in the area and grass cover was sparse. No moist habitats were observed in about 2 km radius. The river bed was sandy and therefore it was not expected to hold water for long periods. *Culicoides* midge collections were made from May the 14th to May the 16th 2010, after the wet season.

Corona

Homestead site (23°23.444´S, 16°09.600´E)

The Corona homestead (1,185 m above sea-level) is situated at the foot of the escarpment. The average slope is gentle and the landscape is slightly undulating. The homestead is surrounded by steep mountains on the North-East. The trap was installed 20 m away from a water trough, which was frequented by horses. Water from an irrigation pipe from a lush garden (15 m away from the trap) seeped into garden-refuse, which laid 5 m from the trap. Sizable lawns were kept in both the front and back garden. *Equus zebra hartmannae* sometimes approached the gardens at the homestead. *Culicoides* midges were collected at this site on February the 14th and 15th and from May the 11th to May the 13th 2010.

Veld site (23°24.194´ S, 16°10.907´ E)

The corresponding veld site (1,192 m above sea-level) is situated on a gentle hill, which lies among mountains, 3 km from the homestead site. There were no permanent surface waters in the immediate surroundings, but several large natural pools and one earthen dam was observed roughly 5 km away. Rain had fallen 2 days prior the records being taken. Several moist habitats were found in about a 10 km radius of the trap. Many hollows were still moist and many rock pools held water. *Culicoides* midge collections were made during the rainy season, on February the 14th and 15th 2010 and after the principal rainy season, from May the 11th to the 13th 2010.

Sampling equipment and collection methods

Onderstepoort 220V suction UV-light traps (24) were installed at farm homesteads and comparative veld sites to collect the *Culicoides* midges. At all the sites, traps operated at roughly the same height above the ground (1.7 m) and with as similar exposures to their surroundings as possible.

The traps operated from sunset to sunrise, regulated and synchronised by the use of a Toptronic® programmable time switches (model TDDT7) (SolarMAX, Kleinmond, Western Cape, South Africa). At the veld sites, light traps were powered by a generator with the power output equal to that of the mains electricity by which the homestead traps were powered. The specimens were collected and preserved as described by Becker (4). The *Culicoides* midges in all collections were counted and identified to species level.

Collections made at each of the 4 homestead sites (*n*=20) were compared to the nearby veld site (*n*=20). The results obtained from the 3 closely related veld sites at Hureb Süd were grouped to facilitate comparisons. The Wilcoxon Matched Pairs Test was used to determine statistical significant differences between the total *Culicoides* midges collected from corresponding homestead and veld sites per farm and between the total collected from homestead and veld site across all farms. Only the most dominant species present in collections from either the veld or the homestead site on a farm were used in the analysis.

Results

Forty light trap collections were made between February the 27th and October the 6th 2010. The overall numbers of individuals of each *Culicoides* species collected at the 4 farms' corresponding homestead and veld sites is summarised in Table I.

A total of 10,178 *Culicoides* midges were collected in the 20 collections made at the homestead sites compared to only 1,733 individuals collected at the 20 veld sites (Table I). Significantly more *Culicoides* midges were collected at the homestead than at the veld sites (Z=2.0226. P=0.043).

At Hureb Süd, statistically significant more *Culicoides* midges (3,706) were collected at the homestead site than at the veld site (462) (Z=2.023, P=0.043). It was the same for the collection made at Isabis (Z=2.023, P=0.043), where 5,961 *Culicoides* midges were collected at the homestead site compared to only 8 collected at the veld site. The higher number of *Culicoides* midges collected at the Neu Heusis veld site (445) was not statistically different from that at the homestead site (393) (Z=0.405, P=0.686). At Corona, the higher number of midges collected at the veld site (818) compared to that collected at the homestead (118) was borderline significant (Z=1.753, P=0.080).

Midges belonging to 31 species were collected from all the sites. Twenty-eight species were collected from the veld sites and 25 species were collected from the homestead sites, 22 of which were present both at homestead and veld sites. Overall, *Culicoides ravus* was the dominant species at both the homestead and veld sites.

The confirmed vector of AHS, *C. imicola*, was not only 91% represented at homestead sites, but also represented 19.6% and 10.8% of the all *Culicoides* midges collected at the homestead and veld sites, respectively. The proportional representation of *C. imicola* was significantly higher at the homestead sites than at the veld sites (p<0.001). The Corona homestead and veld sites each contributed only 1% of the total of the collected *C. imicola*, whereas Hureb Süd and Isabis homesteads respectively contributed 44% and 45% to the total of the collected *C. imicola*.

Discussion and conclusions

Onderstepoort 220V suction UV-light traps, or similar models, are used routinely worldwide to determine the abundance and the occurrence of *Culicoides* midges (1, 7, 9, 22). As with any sampling methods light traps have certain shortcomings. They have a relatively short attraction range of about 2-4 m radius (25) and thus they only sample *Culicoides* midges at a relatively fine scale. The consistency of the fraction of the *Culicoides* population within this attraction range collected is influenced by the trap's height above ground level (23) and by the environmental conditions - such as host densities, local variations in air temperature, moisture and wind strength - which may affect the fine-scale distributions of *Culicoides* midges in immediate surroundings of the traps (26). Despite a great variety of factors that can influence the numbers of *Culicoides* midges collected with light traps, this is still regarded as the most reliable and practical way to determine vector presence and abundance in a given area (24).

Notwithstanding the shortcomings of the collection method, it is clear that overall number of *Culicoides* individuals collected was significantly greater at homestead sites than at veld sites. The overwhelmingly higher numbers collected at the homestead sites at Isabis and Hureb Süd compared to the veld sites highlight the need to identify the factors that may be responsible for this phenomenon. Further research should investigate to what degree and manner various anthropogenic activities may contribute to the proportional

Table I. Culicoides *species and the total of each species collected with light traps at homestead and veld sites located at 4 farms in the South-Western Khomas Region between February and October 2010.*

Farm name	Neu Heusis		Hureb Süd		Isabis		Corona		Total collections	
Site	House	Veld	House	Veld	House	Veld	House	Veld	House	Veld
No. collections	6	6	6	6	3	3	5	5	20	20
No. of species	12	20	17	19	12	2	13	14	25	28
Culicoides species										
C. ravus	150	229	1,331	201	2,681	-	50	552	4,212	982
C. imicola	44	59	951	98	983	-	19	30	1,997	187
C. bolitinos	-	2	15	6	-	-	-	-	15	8
C. pycnostictus	125	58	285	72	780	7	8	23	1,198	160
C. subschultzei	29	37	471	6	485	1	10	160	995	204
C. leucostictus	29	18	56	25	463	-	2	1	550	44
C. exspectator	5	4	453	22	24	-	-	-	482	26
C. pretoriensis	-	15	5	8	24	-	13	19	42	42
C. tropicalis	3	1	59	1	245	-	-	6	307	8
C. herero	-	3	13	1	-	-	5	9	18	13
C. bedfordi	-	2	26	1	-	-	3	3	29	6
C. tuttifrutti (#30)	-	1	-	7	52	-	3	-	55	8
C. nivosus	1	-	4	1	221	-	-	-	226	1
C. schultzei	-	4	12	-	-	-	-	-	12	4
C. olyslageri	1	4	-	-	-	-	-	7	1	11
C. accraensis group	4	-	2	-	-	-	-	4	6	4
C. similis	1	2	-	-	2	-	-	-	3	2
C. brucei	1	-	-	-	-	-	1	2	2	2
C. neavei	-	-	-	1	-	-	1	1	1	2
C. macintoshi	-	1	-	1	1	-	-	-	1	2
C. #89	-	-	-	2	-	-	2	-	2	2
C. punctithorax	-	1	-	1	-	-	-	-	0	2
C. #50	-	-	12	-	-	-	-	-	12	-
C. trifasciellus	-	-	12	-	-	-	-	-	12	-
C. nr albopunctatus	-	-	-	8	-	-	-	-	-	8
C. cornutus	-	2	-	-	-	-	-	-	-	2
C. nigripennis group	-	1	-	-	-	-	-	-	-	1
C. #61	-	1	-	-	-	-	-	-	-	1
C. #94	-	-	-	-	-	-	1	-	1	-
C. #33	-	-	-	-	-	-	-	1	-	1
Total	393	445	3,706	462	5,961	8	118	818	10,178	1,733

higher occurrence of *C. imicola* and other species collected at the homestead sites during the present survey. Very little is known about the general biology, the transmitted diseases, and the vector competences for most of these species. This could have implications for the management of these vector-borne diseases. Seasonal and more extensive surveys are required to pinpoint subtle differences in distribution and abundance of *Culicoides* midges at various veld sites to ascertain whether potential *Culicoides* vectors could be present during, for instance, the drier winter months (4). The rather limited current veld collections made at Hureb Süd suggest that this may indeed be possible, especially in ephemeral river valleys where some of the veld collections at Hureb Süd were made.

However, the numbers of *Culicoides* midges collected at the Corona veld site was greater than the one collected at the homestead site (Table I), indicating that more factors, variable across a landscape, may contribute to variation in homestead/veld abundances, such as host densities, wind strength, local temperatures and available moisture sources. Available moisture (humidity and soil moisture) is linked to precipitation, but also to topography, vegetation cover and soil type, which determines an area's ability to store moisture. Lower-lying areas with less porous soils, for instance, are generally

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associated with better moisture retention (14). It is noteworthy that, although the total *Culicoides* midges collected at the Corona veld site was relatively high, the number *C. imicola* individuals collected was the lowest among all sites. This was the case of both the Corona homestead and veld site.

The most abundant species at both the homestead and veld sites in the present survey was *C. ravus*. Based on its localised and low overall abundance, as found in a country-wide light trap survey in South Africa, its potential as an Orbivirus vector was considered to be low (19). It was, nonetheless, shown that this species will take a blood meal from horses (14) and because the relative abundance of this species in the Khomas Region is high, it may act as a vector of vector-borne diseases such as AHS.

Due to the fact that the homestead sites generally supported larger numbers of *Culicoides* midges than the veld sites, it is proposed that these anthropogenic impacted sites have created favourable 'islands' which support greater populations of *Culicoides* midges than could otherwise have been found in the area. This has implications for the range and distribution of *Culicoides*-vectored diseases such as AHS into areas where they may otherwise have been limited by the natural environmental conditions.

It is concluded that *Culicoides* species distribution and/or activity are probably boosted by the favourable conditions provided by anthropological activity. The winter circulation of *Culicoides*-vectored diseases, such as AHS, is also expected to be limited to these sites. An increase in anthropogenic activity and impacted habitats in the South-Western part of the Khomas Region may also increase the risk of outbreaks of *Culicoides*-borne diseases.

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