3. RESULTS

3.1. EXPERT OPINION ON AFRICAN HORSE SICKNESS IN THE SOUTH-WESTERN KHOMAS REGION AND THE GEOGRAPHICAL CHARACTERISTICS OF THE STUDY AREA

3.1.1. Introduction

The current study area was remote and very few published detailed descriptions of the area were available. Records of AHS were not well kept and weather stations that were regularly monitored were few and very far apart, so that they could not describe the rainfall accurately to the scale of the current study area. It was therefore considered important to explore the area in more detail and to describe the occurrence of AHS in horses, by use of expert opinion. The opinions of local farmers were regarded as expert opinion for the purposes of this study (see the list of names in Table 3.1).

The Namibian escarpment is known for its arid and sparse vegetation (Mendelsohn *et al*., 2002). One would not expect to find the AHSV in this dry and mountainous environment, which is expected to restrict the occurrence of the insect vectors (Conte *et al*., 2007) and hosts of the AHSV. Indeed, historically, some farms in the Khomas Hochland and Gaub River area were used as quarantine sites for horses, yet recently outbreaks of the disease have been reported in the area. It is unclear whether or not these outbreaks originated from a ‘natural’ nidus of infection or whether or not faulty applications of the AHS vaccines might have caused some of the outbreaks. However, *E. z. hartmannae* adapted to dry, mountainous areas (Cillié, 2004) reportedly roam free in this area, and might have acted as cycling hosts.

It was therefore the object of this study to explore the occurrence of AHS and the variables that might affect it, such as the geographic (environmental) characteristics of the south-western Khomas Region, the management of horses and vaccine practices.

3.1.2. Materials and methods

3.1.2.1. The study area

Twenty-four farms (each covering an area of about 13 000 ha) were identified to be sampled along the selected study transect discussed under section 2.1 (line indicated in blue in Fig. 2.2 and Fig. 3.1). As seen in Fig. 3.1, the study transect represents a rainfall gradient. A more detailed rainfall distribution was required for the scale of the study area size. This is also the area in which the hosts and vector were sampled (see sections 3.2 and 3.3).

At most farm sites, the relief is very steep and in general the study area is characteristically mountainous (Mendelsohn *et al.,* 2002). In the south-western Khomas Region, *E. z. hartmannae* were numerous (Mendelsohn *et al.,* 2002) and potentially the principal AHSV reservoir in the area (Barnard, 1998; Coetzer & Guthrie, 2004). Their distribution will be discussed in more detail in section 3.2.

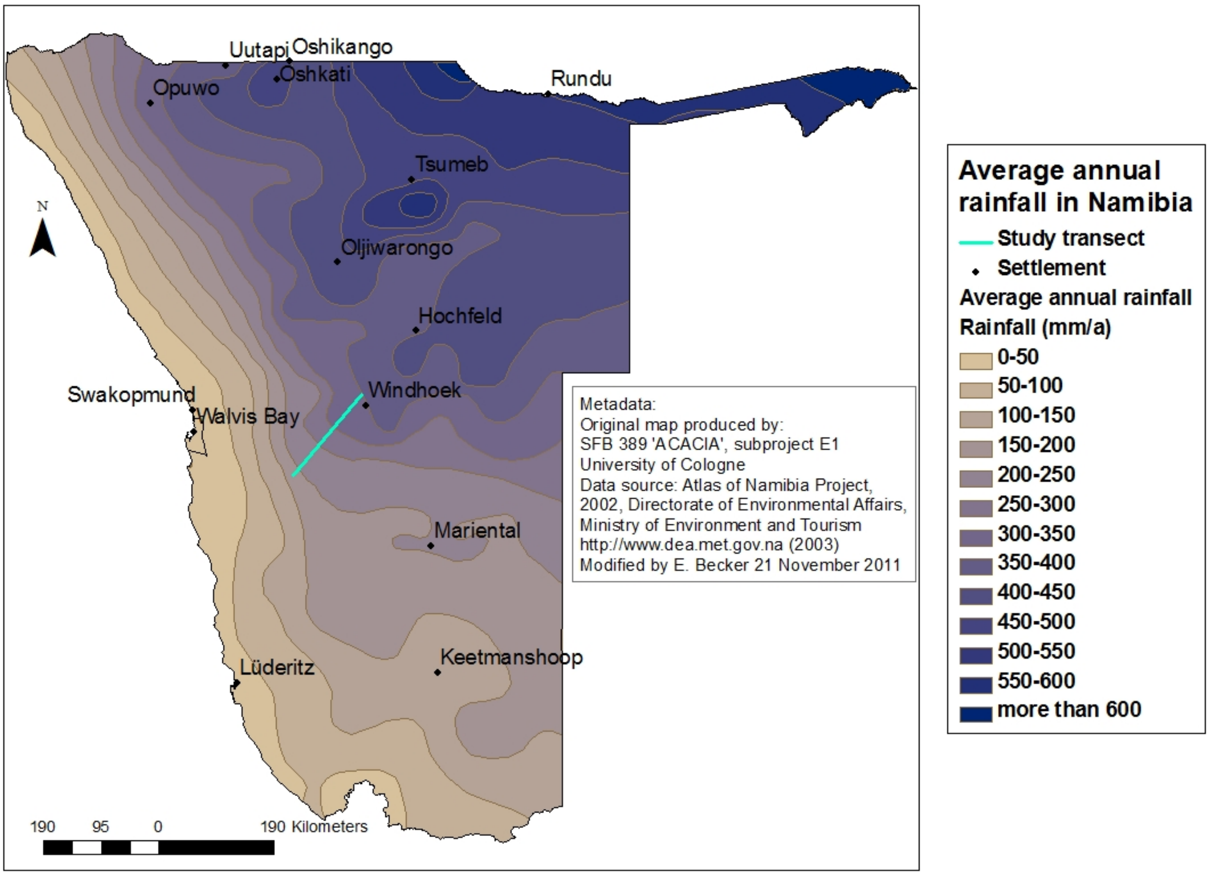


Figure 3.1. Mean annual rainfall zones of Namibia

Adapted from the Atlas of Namibia Project (Anon, 2002) (see also Mendelsohn *et al.*, 2002))

3.1.2.2. The collection of expert opinion by questionnaires

For identification of the study area, a mean annual rainfall map of Namibia was used, provided by Mendelsohn *et al*. (2002). An adapted version of the map is shown in Fig. 3.1. The original shape files plus attribute information is provided online by the Atlas of Namibia Project (2002) by the Directorate of Environmental Affairs, Ministry of Environment and Tourism.

Along the selected study transect discussed under section 2.1 (line indicated in blue, Fig. 2.2 & Fig. 3.1), farms were identified by use of a topographic map obtained from the land surveyors in Windhoek. All the farms identified on this gradient line for which contact numbers were available, were sampled. The owners in question were contacted telephonically to request their assistance in the study.

Each farmer was asked a series of questions concerning their opinion on the state of AHS and the geographical characteristics of the area. A detailed summary of the questions is contained in 3.1.2.2 (a) below. The responses by the farmers were interpreted and the questionnaire completed by the investigator. The questionnaire consisted mostly of closed-ended and absence/presence questions. Open-ended questions covered qualitative data and required succinct, descriptive responses. Intensity-scaled questions were used to standardise responses such as density of plant growth.

Farmers were contacted and interviewed at three different periods for this purpose. The first interview was conducted in March 2009 and follow-up interviews were conducted during November 2010 and October 2011, to obtain some measure of variation to the mean annual rainfall data (see 3.1.2.3) and cases of AHS as reported in the March 2009 survey.

Questions also covered the numbers and movements of the cycling host, *E. z. hartmannae*, as will be discussed in section 3.2.

The questionnaire results were taken into consideration when interpreting the occurrence of AHS, its host and vectors. As the data is subjective, the results indicate directions for further study and research.

(a) Questionnaire contents

The responses to these questions are summarised in Table 3.1 to Table 3.3.

(i) What is the nature of the available electricity supply on the farm?

(ii) Rainfall:
- What is the estimated mean annual rainfall of the farm?
- What was the highest rainfall month(s)?
- What was the rainfall over the season 2009/2010?
- What was the rainfall over the season 2010/2011?

(iii) Surface water (possible *Culicoides* species. breeding sites):
- Are there any permanent bodies of water on the farm?
- Presence of ephemeral pools – how long do they hold water?
- Are there any irrigation practices and/or watered gardens?

(iv) Vegetation:
- Is it typically ‘grassland’ or ‘shrubland’?
- If ‘shrubland’ would you classify it as: dense, medium-dense or sparse?

(v) Horses:
- Do you own horses, if ‘yes’ – how many?
- Are the horses stabled or are they free-roaming?
- Are they vaccinated against AHS?
- How regularly are the horses vaccinated?
- Are vaccinations preventative (due to a history of AHS on the farm) or precautionary (due to rumours of AHS in the area)?

(vi) AHS in horses
- How many cases of AHS occurred in the past five years?
- How many cases of AHS occurred during 2009/2010?
- How many cases of AHS occurred during 2010/2011?
- In which months did cases of AHS occur?
- Is AHS considered a problem on the farm?

(vii) Other possible *Culicoides* species hosts:
- Do you own cattle - how many per hectare?
- Are there donkeys or mules in the area?
- Are there other large mammals in the area e.g. wildebeest, eland?
- Are there Hartmann’s mountain zebra in the area? (see 3.2.2.2)

3.1.2.3. Estimated long-term annual rainfall in the south-western Khomas Region

For an indication of the possible variation in annual rainfall and possible associated cases of AHS, a 38-year rainfall record of Isabis farm was obtained from the owners, see Fig. 3.5. This long-term data served as an estimate and extrapolation of the nature and variability of rainfall typically experienced in the south-western Khomas Region and therefore an indication of the degree to which the occurrence of AHS, its hosts and vector may vary from what this survey indicated.

The farmers were asked to provide the number of AHS related horse mortalities from which incidence proportion was calculated, in the last five years (see 3.1.2.2(a)). However the periods of low and high rainfall may span over a longer period than five years. There was a chance that the survey would represent either of the extreme cases, rather than the mean AHS incidence proportion.

The follow-up questionnaires conducted during November 2010 and October 2011 (see 3.1.2.2) were used to test the trend suggested by the 38-year rainfall record from Isabis farm and whether or not such variation is seen in more farms across the south-western Khomas Region. The possible related occurrence of AHS incidence proportion was also recorded and compared between the two years.

3.1.2.4. Data analysis

(a) Expert opinion questionnaire summary and standardisation of responses

The questionnaire was conducted telephonically and responses by the farmers were therefore interpreted and recorded by the investigator. For ease of interpretation, some responses to questions were standardised to be expressed in the same format.

Densities of cattle, as reported by respondents (farmers), were standardised to head of cattle per 10 hectares of farmland. Incidence proportion (Rothman & Greenland, 2005) was used to standardise the occurrence of cases of AHS in the past five years (2004 to 2009), in 2009/2010 and 2010/2011, as horse population sizes were variable among sites (Table 3.2, Fig. 3.4).

Incidence proportion (%) = ('Number of cases of AHS over a certain period'/Population at t_0) x 100%, where population at the start of the period, $t_{0,}$ was determined by adding the current horse population size, to the number of horses that have died due to AHS. The t_0 population did not take into consideration death by other causes and other alterations to herd numbers such as the buying and selling of horses over the course of the period. However, farmers reported that their horse populations have remained approximately the same over the five-year period.

The answers were tabulated on a Microsoft Excel ® a spreadsheet (see 3.1.3.1, Tables 3.1 to 3.3).

The incidence proportion per site was expressed graphically as pie charts in context with their geographical situation. This is discussed in 3.1.2.4 (c).

(b) The relationship between the occurrence of AHS in horses, horse population size and annual rainfall in the south-western Khomas Region

Regression techniques were used to describe the effect of horse population size on the number of cases of AHS and the effect of annual rainfall on the occurrence of AHS incidence proportion. The relevant data from the questionnaire discussed in 3.1.2.2 was used for these analyses.

(i) The regression between the horse population at t_0 and the number of cases of AHS indicated whether or not incidence proportion was an acceptable means of standardising the occurrence of cases of AHS across the study area. The number of horse mortalities (cases of AHS) reported by farmers (see Table 3.2) was plotted against the horse population at t_0, by use of the STATISTICA software of StatSoft Inc. The p-value, correlation coefficient (r) and the coefficient of determination (r^2) were calculated by use of the same software.

(ii) The same procedure was applied as (i) above to determine the relationship between the occurrences of AHS incidence proportion and annual rainfall for the years 2009/2010 and 2010/2011. The results should be viewed in support of the spatial representation of the relevant data of the questionnaire discussed in 3.1.2.4 (c).

(c) Spatial representation and interpolation of questionnaire data

Geographic Information Systems (GIS) were used to plot and illustrate patterns of occurrence in AHS incidence in context with its geographical situation with certain environmental variables.

The area topography was drawn by obtaining altitude information by remote sensing from the Google Earth™ application. Altitude points were taken at equal increments over the study area and the corresponding altitude was recorded. The Google Earth™ background raster was imported into the ESRI® ArcMap™ software and a corresponding feature point shapefile was constructed with altitude as attribute z-value.

Co-ordinates for some of the sampled farms were determined by a Garmin® global positioning system and imported into ESRI® ArcMap™ software as a feature point shapefile. Data from each farm obtained by the questionnaire was read into the attribute table for each feature point. Mean annual rainfall was used as the feature z-value.

ESRI® ArcGIS® software was used to calibrate all data layers to the same geo-reference, using the World Geodetic System 1984 (Dana, 1995) as the co-ordinate system.

Point data was interpolated over the entire study area. Algorithms provided by the Spatial Analyst extension of ESRI® ArcGIS® software was used to predict unknown values of intervening space based on the data point values of the sampled farm sites and their distribution. The interpolated values were expressed in raster format.

Kriging (Wescott & Brandon, 1999) was used to interpolate rainfall and altitude data points. From the rasters generated, isohyets and contours were constructed by use of the Spatial Analyst extension of ESRI® ArcGIS® software. The results were summarised in Fig. 3.4.

The incidence proportion of AHS per site was plotted in their geographical location as feature point data, expressed as pie charts. AHS related horse mortalities were expressed as a fraction out of the t_0 horse population (see 3.1.2.4(a)).

(d) Presentation of long-term annual rainfall in the south-western Khomas Region

Long term variation in rainfall in the area was indicated by the annual rainfall recorded at Isabis farm, where the 38 year rainfall data was obtained. The monthly data was entered in table form into the Microsoft Excel ® software (Appendix 1) and the annual totals calculated. The rainfall periods stretched from October to March of the following year. The rainfall is recorded for when the rainfall period ends, i.e. the rainfall period 2008/2009 is recorded as 2009. The mean annual rainfall was calculated from these annual totals. A bar graph was drawn for the annual totals for each year, together with the mean annual rainfall, expressed as a line. The years which have above average rainfall were expressed as those bars which extend past the average line and the years that received below average rainfall were expressed as bars that did not extend to the level of the mean annual rainfall line (Fig. 3.5).

3.1.3. Results

The questionnaire data obtained from telephonic interviews of farmers were summarised in Table 3.1 to Table 3.3 and represents subjective data pertaining to the physical description of the study area, AHS hosts, vaccination practices and AHS related horse mortalities in the south-western Khomas Region. Fig. 3.2 and Fig. 3.4 were drawn from the information in Table 3.1 and Table 3.2. Fig. 3.4 illustrates trends on expert opinion of AHS and mean annual rainfall. Fig. 3.3 is drawn from the information presented in Table 3.3. Table 3.3 will also be used to describe the possible variation from the mean annual rainfall in the south-western Khomas Region, along with Fig. 3.5 in 3.1.3.4.

3.1.3.1. AHS and the geography of the south-western Khomas Region

Table 3.1 contains information from respondents (farmers) from the first questionnaire interview made in March 2009, which included questions about the physical description of the study area. Table 3.2 is a continuation of the questionnaire, as described in 3.1.2.2(a), but contains the responses pertaining to AHS hosts, vaccination practices, AHS related horse mortalities and the calculated incidence proportion of AHS as described in 3.1.2.4.

Table 3.3 summarises the follow-up questionnaire conducted in November 2010 and October 2011, which indicated the variation to the mean annual rainfall and the occurrence of AHS reported in the south-western Khomas Region in Table 3.1 and Table 3.2. The data from Table 3.3 will also be used in the determination of the relationship between the occurrence of cases of AHS in horses and the rainfall an area receives in a year (see Table 3.5 and Fig. 3.3)

The mean annual rainfall in the area was reportedly low in general (Table 3.1) according to the March 2009 survey, the highest rainfall recorded was at Neu Heusis (420 mm/a) and the lowest at Corona and Weenen (120 mm/a). February was reported as the highest rainfall month (Table 3.1). At all the locations, pools and dams were reported as holding water for most of the year (Table 3.1). The vegetation cover is mostly sparse to medium dense (Table 3.1).

Horses are generally not stabled, but kept in camps around the homestead area (Table 3.2). All of the animals that had died from AHS were young. The greatest number of cases of AHS for the last five years was recorded at Farm Hochland (15 cases), Hureb Süd (14), Heusis (13), Claratal (12) and Tsauwasis (8). The AHS incidence proportion ranges from zero to 40%. The highest incidence proportion was recorded at Tsauwasis at 40% of the population infected with AHS in five years. Other areas with high incidence proportion are Farm Hochland (33%), Heusis (30%), Kobos (23%) and Hureb Süd (17%).

Eleven out of the 24 farmers (46%) vaccinate their horses, of which 9 (38%) do so regularly (Table 3.2) and the other 8% vaccinate occasionally. Of the farmers who vaccinated their horses, 34% do so as a precautionary measure and not because they have suffered heavy losses in the recent past. Cattle were kept, on average, at about one head of cattle per 10 hectares (Table 3.2). Donkeys and mules were also few, on average, six were kept on a farm of approximately 13 000 ha in size.

The farm that received the highest rainfall in 2009/2010 was Neu Heusis at 270 mm and the lowest rainfall was received at Jonkersgraf at 40 mm (Table 3.3). The rainfall was, on average, 112 mm below the mean annual rainfall received per farm (Table 3.3). Kiamsab, Weissenfels and Jonkersgraf had the largest variation from the mean, with rainfall more than 200 mm below their rainfall averages. Kobos, Hureb Süd, Corona and Hakos, received less than 50 mm below mean annual rainfall and therefore rainfall close to their averages.

Over the period 2009/2010 only two farms reported cases of AHS, Tsauwasis (one) and Hureb Süd (six) (Table 3.3). Accounting for the smaller population of horses at Tsauwasis, the incidence proportion is almost equal at Hureb Süd and Tsauwasis, however Tsauwasis received only 169 mm (Table 3.3) compared with 209 mm received at Hureb Süd. The incidence proportion was 1% on average.

The 2010/2011 rainfall season was at the other extreme where, on average, the mean annual rainfall was exceeded by 626 mm. All the sites except Jonkersgraf experienced two to three times their mean annual rainfall. The 6/10 sites sampled reported cases of AHS in horses. The incidence proportion was 7% on average.

Table 3.1. Questionnaire summary part I: Site, altitude, rainfall conditions, surface water and vegetation of farms in the south-western Khomas Region, recorded in March 2009.

Farm Name	Expert name (Farm owner)	Co-ordinates		Altitude (m)	Estimated annual rainfall	Highest rainfall months	Permanent water	Ephemeral pools		Vegetation type	Vegetation cover
		S	E					Present	Duration (months)		
Hochfels-airy	H. H. Keickebusch			1806	375		No	No	0	Grasslands	.
Aandrus	D.Botha	22° 39.182´	16° 30.010´	1706	250	Feb	Earth dam	Yes	2	Shrubland	Med. dense
Neu Heusis	E. Hoff	22° 36.660´	16° 42.647´	1721	420		Earth dam	Yes	12	Shrubland	Dense
Heusis	Hennings			1697	325	Feb	No	Yes	12	Shrubland	Dense
Groenkloof	W. Esterhuizen			1404	275	Feb	Earth dam	Yes	3	Shrubland	Sparse
Hureb Süd	W. Esterhuizen	22° 29.545´	16° 22.091´	1217	225	Nov-Dec, mid Jan-Feb	3 Earth dams	Yes	12 to 24	Shrubland	Med. dense
Tsauwasis	L. E. Greeff	22° 45.001´	16° 10.201´	1546	285	Jan/Feb	Troughs	Yes	1 to 2	Shrubland	Med. dense
Harmonie	M. Jacobs	22° 46.452´	16° 18.982´	1438	225		Earth dam, pools	Yes	0.75 to 8	Shrubland	Sparse
Jonkersgrab	Bassingthwaighte	22° 54.142´	16° 29.930´	1569	250	Nov/Dec/Jan/Feb	Earth dams	Yes	0.5	Shrubland	Med. dense
Wasserfall Valei	D. Wasserfall	22° 53.657´	16° 22.532´	1369	260	Jan/Feb	Pools	No	0	Shrubland	Dense to sparse
Farm Hochland	H. Rush	22° 58.647´	16° 31.710´	1779	350	Feb/March	Earth dams	Yes	12 to18	Shrubland	Sparse
Kobos	B.D. Gramm	22° 56.300´	16° 10.200´	1310	180		Pools	No	0	Shrubland	Med. dense
Amor	N du Toit	22° 53.700´	16° 6.001´	1378	300	Feb/March/April	Earth dam	Yes	12 to18	Shrubland	Sparse
Landmeister	Meiburgh	22° 41.529´	16° 43.830´	1617	350	Feb	Pools	Yes	1 to 2	Shrubland	Dense
Kiamsab	J. F Schickerling	22° 58.683´	16° 24.602´	1213	325	Feb/April	Earth dams	Yes	12 to 16	Shrubland	Med. dense
Weissenfels	W. Retief	23° 19.020´	16° 27.008´	1766	375	Feb	2 Earth dams	Yes	3	Shrubland	Sparse to med. dense
Chaibis	Bredenkamp			1200	165		Springs	No	0	Shrubland	Sparse
Corona	U. Barth	23° 23.476´	16° 9.591´	1182	120		None	Yes	3 to 4	Shrubland	Sparse
Swartkrans	I. Oosthuizen	23° 33.401´	16° 10.800´	1777	150	Jan/Feb/March/April	Earth dam	Yes	12	Shrubland	Sparse
Natas	H. Schurz	23° 11.002´	16° 17.600´	1580	210	Feb/March/April	Troughs, pools	Yes	6	Shrubland	sparse
Isabis	J. Cranz	23° 25.398´	16° 30.608´	1786	220		Earth dams	Yes	6	Shrubland	Sparse
Hakos	W. Straube			1626	250	Feb	2 Earth dams	Yes	Unknown	Shrubland	Sparse
Weenen	v Heerden	23° 25.185´	16° 15.601´	1566	120		Pools	Yes	4	Shrubland	Sparse to med. dense
Claratal	H. Frier	22° 47.827´	16° 50.608´	1947	310	Jan/Feb		Yes	10	Shrubland	Sparse

Table 3.2. Questionnaire summary part II: Number of horses, livestock, cases of AHS in horses and AHS vaccination practices in the south-western Khomas Region, recorded in March 2009.

Farm Name	**Number of horses**	**AHS-cases the in past 5 years**	**Estimated incidence propotion (%)**	**Horses kept at stable**	**Vaccinate**	**Horse age at contracting AHS (years)**	**Precautionary vaccinations**	**No. Cattle per 10ha**	**Donkeys**	**Mules**
Hochfels-airy	30	0	0	Yes	Yes	1 to 2	Y	0.7	8	2
Aandrus	20	0	0	No	No	.	.	0.7	0	0
Neu Heusis	45	3	6	Occasionally	Yes	Unknown	Y	1	5 or 6	0
Heusis	30	13	30	No	Some horses	Unknown		1	2	0
Groenkloof	0	0	.	No	.	.		0	2	0
Hureb Süd	70	14	17	No	Yes	1 to 2	N	0.7	5 or 6	0
Tsauwasis	12	8	40	No	Yes	Unknown	N	0.9	5	0
Harmonie	Many: number unknown	2	.	No	No	Unknown	.	1	5	1
Jonkersgrab	15	0	0	No	No	.	.	0.3	10	0
Wasserfall Valei	18	0	0	No	No	.	Y	0.8	6	0
Farm Hochland	30	15	33	No	Yes	1 to 2	N	.	0	0
Kobos	20 (Desert horses)	6	23	No	No	4	N	0.8	0	0
Amor	20	0	0	No	Occasionally		.	0.8	15	3
Landmeister	35	0	0	No	No	.	N	0.4	2	0
Kiamsab	40	7	15	No	Yes	1 to 2	Y	0.6	5 or 6	0
Weissenfels	120	7	6	Yes	No	1 to 2	Y	1.8	0	0
Chaibis	23	0	0	No	Yes	.	Y	0.1	5	0
Corona	50	0	0	No	No	.	N	0	20	0
Swartkrans	8	0	0	No	Yes	.	Y	0	0	0
Natas	15	1	6	No	Yes	2	Y	0.2	5	0
Isabis	16	0	0	No	Yes	.	Y	0.7	20	0
Hakos	10	0	0	No		.	Y	0.5	0	0
Weenen	5	0	0	No	Yes	.	N	4	10	0
Claratal	70	12	15	Yes	Yes	1 to 2	Y	0.6	0	0

Table 3.3. The mean annual rainfall received and cases of AHS on farms in the south-western Khomas Region during 2009/2010 and 2010/2011, survey conducted in November, 2010 and October 2011.

Farm Name	**Estimated mean annual rainfall (mm/a)**	**Number of Horses**	**2009/2010 Rainfall (mm$_{09/10}$)**	**2010/2011 Rainfall (mm)**	**2009/2010 rainfall deviation from mean (mm$_{09/10}$ - mm/a = mm$_{deviation}$)**	**2009/2010 rainfall deviation from mean (mm$_{10/11}$ - mm/a = mm$_{deviation}$)**	**No. of AHS-cases 2009/2010**	**No. of AHS-cases 2010/2011**	**2009/2010 Incidence proportion (%)**	**2010/2011 Incidence proportion (%)**
Aandrus	250	20	180	1000	-70	750	0	2	0	10
Neu Heusis	420	45	270	1550	-150	1130	0	2	0	4
Hureb Süd	225	70	206	880	-19	655	6	0*	9	0
Tsauwasis	285	12	169	690	-116	405	1	1	8	9
Jonkersgrab	250	15	40	200	-210	-50	0	0	0	0
Wasserfall Valei	260	18	89	1250	-171	990	0	2	0	11
Kobos	180	20	170	no data	-10	no data	0	no data	0	no data
Landmeister	350	35	157	1000	-193	650	0	0	0	0
Kiamsab	325	40	90	1000	-235	675	0	10	0	25
Weissenfels	375	120	150	850	-225	475	0	17	0	14
Chaibis	165	23	100	no data	-65	no data	0	no data	0	no data
Corona	120	50	80	700	-40	580	0	0	0	0
Swartkrans	150	8	80	no data	-70	no data	0	no data	0	no data
Hakos	250	10	206	no data	-44	no data	0	no data	0	no data
Weenen	120	5	54	no data	-66	no data	0	no data	0	no data
Average	248	33	136	912	-112	626	0.5	3	1	7
Standard deviation	89	29	63	339	76	306	1	5.5	3	8

* Hureb Süd's owner applied insect-repellent extensively during 2010/2011

3.1.3.2. The relationship between the occurrence of AHS in horses, horse population size and the mean annual rainfall in the south-western Khomas Region

Table 3.4 shows the result of regression with horse population size at t_0 as the independent variable, and cases of AHS as the dependent variable. It was determined whether or not there was a significant positive relationship between horse population size and the number of cases of AHS. The input data for this regression analysis was from the estimated values provided by expert opinion (as defined in 3.1.1), see Table 3.1 and Table 3.2. Fig. 3.2 shows the scatter plot of horse population at t_0 and the number of cases of AHS obtained from Table 3.2.

Fig. 3.2 displays a positive correlation between the horse population at t_0 and the number of cases of AHS amongst horses. From Table 3.4, the regression coefficient, $b^* = 0.11$. The correlation coefficient between the two variables, $r = 0.6$. The coefficient of determination, $r^2 = 0.36$ (Fig. 3.2); that is 36% of the variation in cases of AHS was expleained by the linear regression. The greatest frequency of samples taken had a horse population of between four to 44. There is a linear relationship between the number of cases of AHS and horse population size. The results are statistically significant ($p < 0.05$).

Fig. 3.3 shows results of regression analysis with annual rainfall for 2009/2010 and 2010/2011 as the independent variable, and AHS incidence proportion for 2009/2010 and 2010/2011 as the dependent variable. It was determined whether or not the annual rainfall received in an area had an effect on the AHS incidence proportion. Table 3.5 summarises the linear regression constants and statistic attributes. See Table 3.3 for input data for the regression analysis.

Fig. 3.3 displays a positive correlation between annual rainfall and the incidence proportion of AHS for 2009/2010 and 2010/2011. From Table 3.5, the regression coefficient, $b^* = 0.008$. The correlation coefficient between the two variables, $r = 0.57$. The coefficient of determination, $r^2 = 0.32$; therefore 32% of the variation in AHS incidence proportion was explained by the linear regression. The highest frequency of samples were recorded for annual rainfall values between zero to 200 mm. The linear relation between cases of AHS and annual rainfall is statistically significant ($p < 0.05$).

Table 3.4. Regression analysis between the number of cases of AHS and the horse population at t_0

(Refer to Table 3.1 and Table 3.2 for input data).

No. cases (N) = 23	**Correlation coefficient (r)**	**Coefficient of determination (r^2)**	**Y-intercept (b)**	**Regression coefficient (b*)**	**p-value**
Linear relationship and variance	0.60	0.36			
Intercept (b)			-0.07		0.96
Regression coefficient (b*)				0.11	0.002

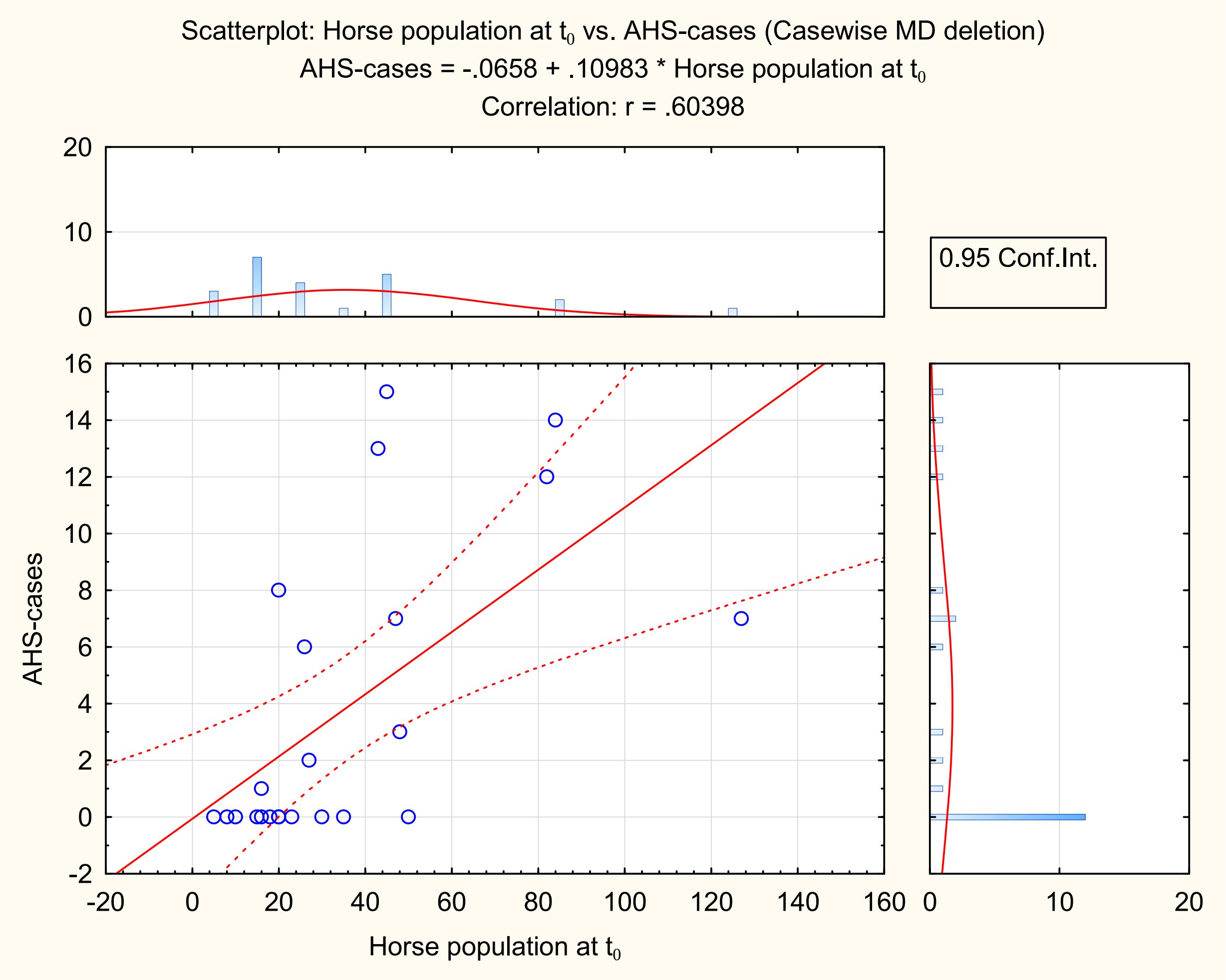


Figure 3.2. Scatter plot of the number of cases of AHS vs. the horse population at t_0 on farms in the south-western Khomas Region for the period 2004 to 2009

(See Table 3.1 and Table 3.2 for input data).

Table 3.5 Regression analysis between AHS incidence proportion and annual rainfall for 2009/2010 and 2010/ 2011 in the south-western Khomas Region

(Refer to Table 3.3 for input data).

No. cases (N) = 24	**Correlation coefficient (r)**	**Coefficient of determination (r^2)**	**Y-intercept (b)**	**Regression coefficient (b*)**	**p-value**
Linear relationship and variance	0.57	0.32			
Intercept (b)			0.26		0.88
Regression coefficient (b*)				0.008	0.004

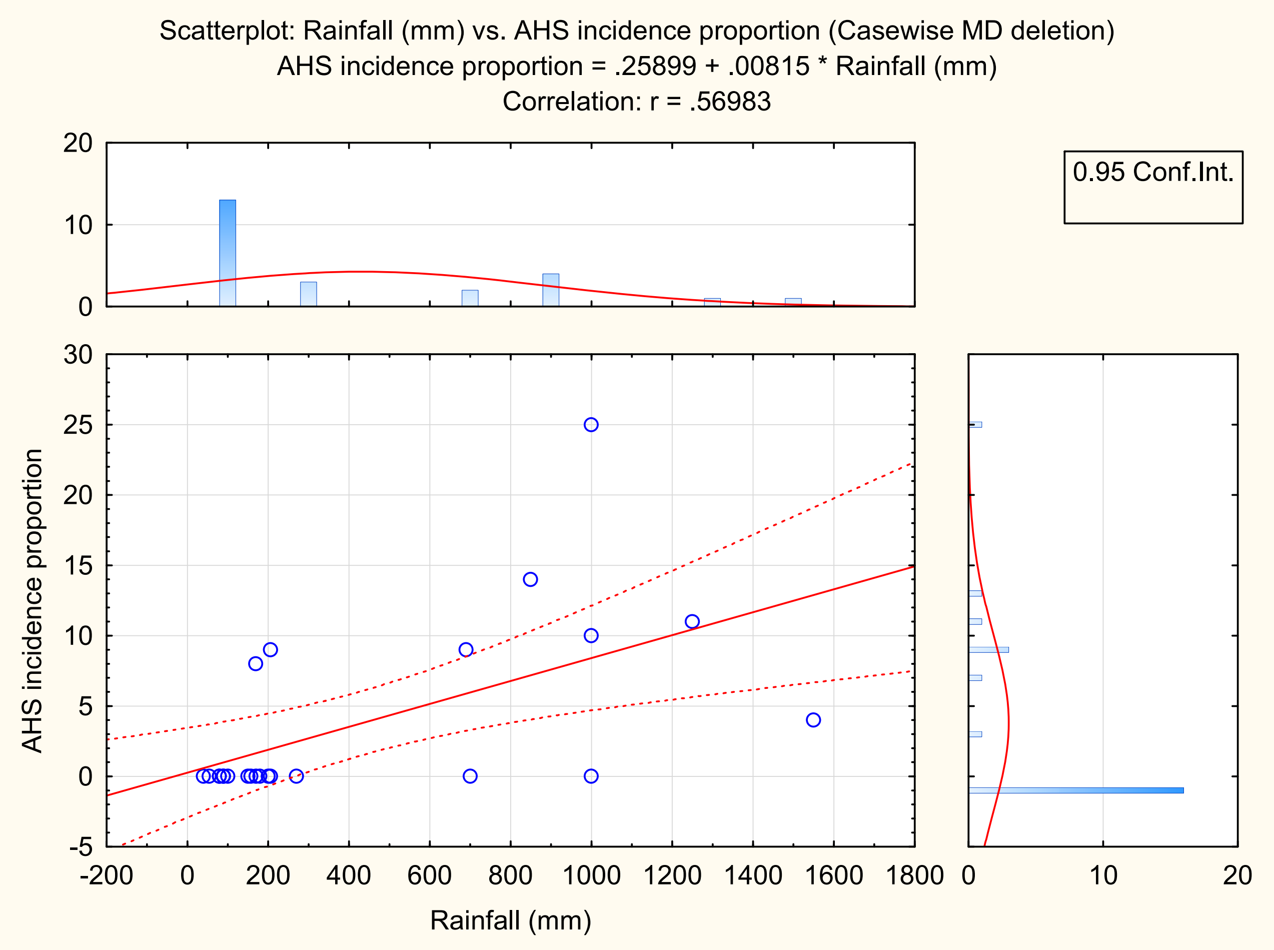


Fig. 3.3 Scatter plot of AHS incidence proportion vs. the annual rainfall for 2009/2010 and 2010/2011 in the south-western Khomas Region

(Refer to Table 3.3 for input data).

3.1.3.3. The spatial representation of questionnaire data

Fig. 3.4 illustrates the trends of the mean annual rainfall and the AHS incidence proportion in the south-western Khomas Region with topography of the area as the setting. The incidence proportion of AHS was plotted as points on their respective geographic position, and was expressed as a proportion of AHS related horse mortalities out of the horse population at t_0 (see 3.1.2.4(a)).

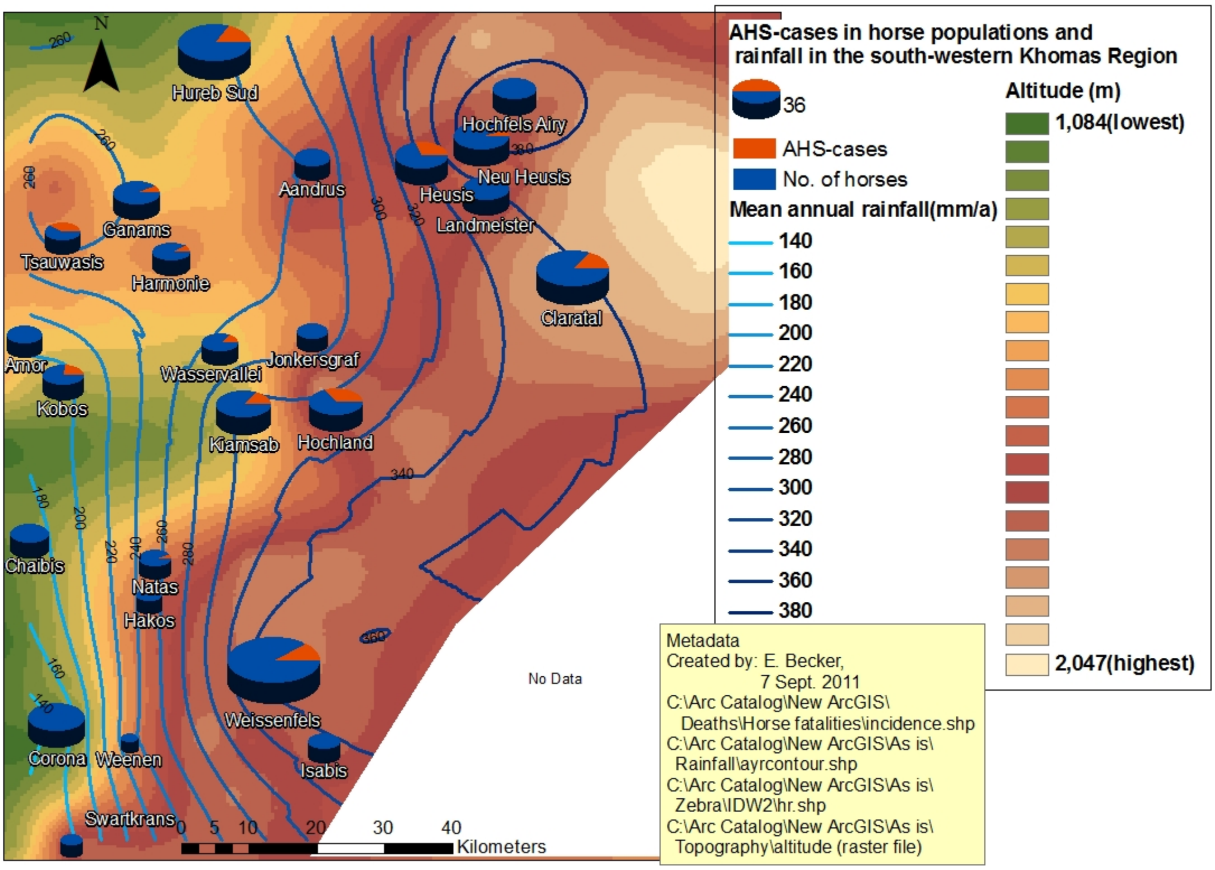


Figure 3.4. Mean annual rainfall and the occurrence of AHS incidence proportion of the past five years in the south-western Khomas Region
(Refer to Table 3.1 and Table 3.2 for input data).

The mean annual rainfall received in the south-western Khomas Region as reported by farm owners, varies from 420 mm/a to 120 mm/a decreasing in general, in a south-westerly direction (Fig. 3.4). The steepest fall in mean annual rainfall was observable between Weissenfels and Corona from east to west. From north to south, the change in rainfall was minimal, from 420 mm/a to 340 mm/a over 100 km. From east to west, from Neu Heusis to Tsauwasis at a distance of 50 km, rainfall first decreased to 260 mm/a but increased slightly to 280 mm/a further west.

Altitude decreased generally from the north-east to the south-western corners of the study area (Fig. 3.4). There is a ridge of higher ground of the Khomas Hochland Mountains, which disrupted a fairly consistent decrease in altitude from east to west. In general, rainfall appeared to be associated with higher altitude and latitude (Fig. 3.4), with a marked decrease in rainfall along the escarpment. However, areas of altitudes 1 200 m above sea level, which are located further north, tended to have higher rainfall values than those of equivalent altitude further south.

More of the AHS related horse fatalities have been reported at higher rainfall areas (Fig. 3.4), with the exception of Hureb, where 14 horses have died of AHS in a rainfall zone of 250 mm/a and Kobos (220 mm/a), where six horses have died of AHS in the past five years (Table 3.2). Both of these sites fell in the mid-rainfall zones of the south-western Khomas Region. Likewise, at the highest rainfall zones, Hochfels Airy, Landmeister and Aandrus, no AHS related horse fatalities were reported. Within the rainfall zones, cases of AHS vary. In the 280 to < 360 mm/a zone, for instance, at five out of nine sites high AHS fatalities were reported, whilst the other four show low to no fatalities. However, below a mean annual rainfall of 150 mm/a, no outbreaks of AHS appear to have occurred in the last five years.

AHS fatalities appear to occur more often at altitudes between 1 300 to 1 750 m above sea level (Table 3.1 and Fig. 3.4). Hureb Süd, which is situated between 1 200 to 1 250 m above sea level, had relatively high incidence proportion compared with other sites of similar altitude. At Weissenfels (1 800 m), the AHS fatalities were high, yet incidence proportion was low. Heusis, Hochland and Claratal recorded high incidence proportion, yet were situated at higher altitudes above 1 750 m.

3.1.3.4. Variation in annual rainfall compared with the mean annual rainfall in the south-western Khomas Region

Fig. 3.5 is a simple bargraph representation of rainfall for the past 39 years at Isabis farm, which is situated in the south-western Khomas Hochland and showed the potential variability in conditions observed during the study period.

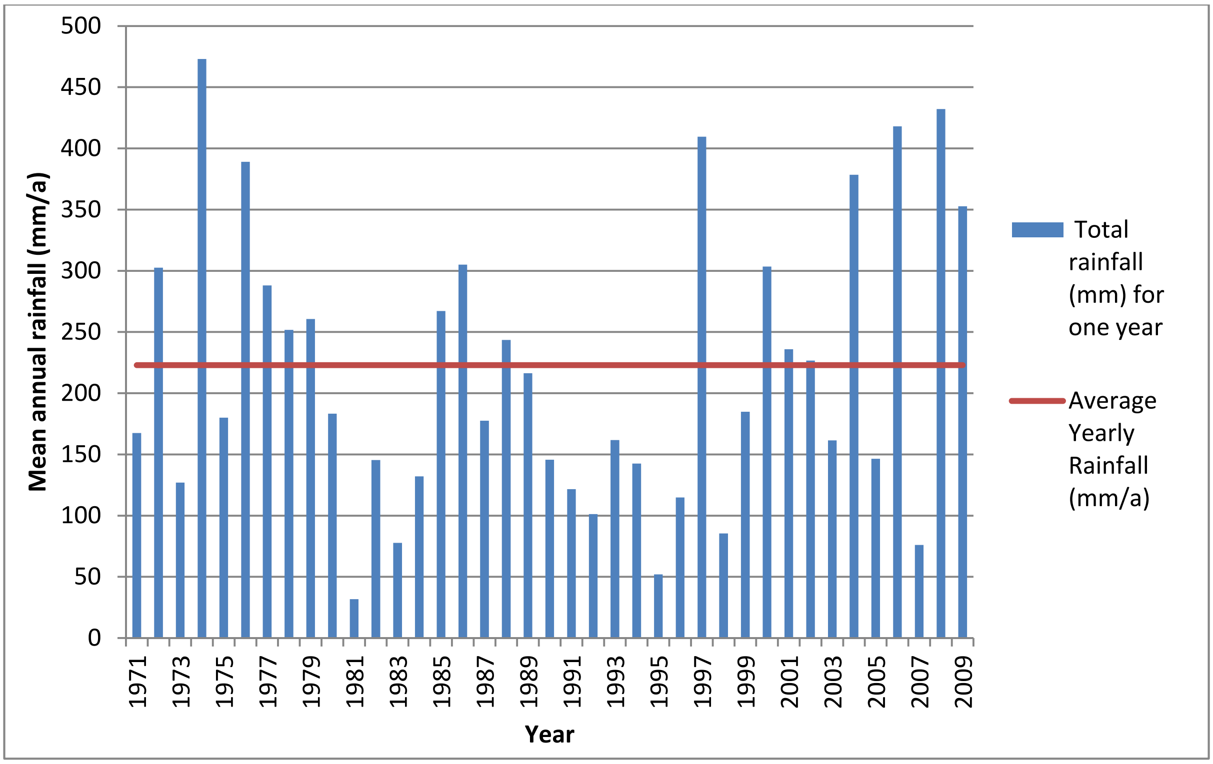


Figure 3.5. Variation in annual rainfall compared to the mean annual rainfall in the past 39 years at Isabis farm
(See Appendix 1)

Fig. 3.5 shows the rainfall at Isabis farm for the past 39 years. The data shows variation in annual rainfall compared with the mean annual rainfall. There is a cyclic pattern of wet and dry periods, with highly variable annual rainfall compared to the mean. There are years that deviated mid-cycle with high rainfall figures, such as in 1997, 2004, 2006, 2008 and 2009 (Fig. 3.5).

3.1.4. Discussion

As expected, the mean annual rainfall in the area was generally low (Table 3.1) and therefore the occurrence of AHS and its *Culicoides* midge vectors were expected to be restricted (Meiswinkel *et al.,* 2004a; Conte *et al.,* 2007). Yet despite the general aridity of the area, AHS was reported (Table 3.2) in the south-western Khomas Region.

From the linear regression in Fig. 3.2, it is concluded that the number of cases of AHS is significantly ($p < 0.05$) positively correlated with the horse population size. Cases of AHS showed a strong linear relationship with horse population size based on a relatively high correlation coefficient of $r = 0.6$. Perhaps in future studies, population size classes can be identified and the number of sites sampled per class be equal in number. A more accurate regression model can then be drawn. Should the horse population size increase, so too should the number of cases of AHS. It is concluded that incidence proportion is an acceptable means to standardise the occurrence of cases of AHS for comparison between sites of variable annual rainfall.

In spite of the limited available rainfall data to accommodate for the effects of variation, the rainfall pattern interpolated from the questionnaire data points (Table 3.1) are congruent with the mean annual rainfall pattern shown by Mendelssohn *et al.* (2002) in Fig. 3.1 of the south-western Khomas Region. Rainfall decreased in two directions in the study area: a small decrease towards the south and sharp decrease in rainfall from east to west.

Therefore it was expected that the greatest AHS incidence proportion would have been found in the north and in the east, decreasing gradually to the south-west (Fig. 3.4), since this is also the pattern expected to be observed in the occurrence of *Culicoides* midges (see 3.3 and 3.3.1.1). However, the actual occurrence of AHS incidence proportion did not follow this pattern.

Investigating the spatial distribution of mean annual rainfall and the occurrence of AHS (Fig. 3.4), it was notable that where the mean annual rainfall was below 150 mm/a, no cases of AHS were reported. However, there appeared to be a low direct relationship between mean annual rainfall and the occurrence of AHS. Within the zone where AHS was reported, its occurrence varied. In the 280 to < 360 mm/a zone, for instance, five out of nine sites reported high AHS fatalities, whilst the other four reported low to no fatalities (Fig. 3.4).

The greatest reported AHS incidence proportion was recorded at neither of the expected locations. In other words, neither the low-lying pediment, nor the higher rainfall sites of the plateau, but along the escarpment and ‘intermediate’ rainfall zones. This is surprising, because *Culicoides* midges which vector the disease are known to be dependent on moisture (Meiswinkel *et al.,* 2004a; Conte *et al.,* 2007). From the linear regression analysis (Fig. 3.3), the data points collected for AHS incidence proportion versus annual rainfall for 2009/2010 and 2010/2011 revealed a significant linear relationship between the variables ($r = 0.57$ and $r^2 = 0.32$). This implies that annual rainfall had an effect on the occurrence of AHS. Further research which included more data points that are more evenly distributed among mean annual rainfall zones/classes are probably required to make definite conclusions regarding the relationship. Other variables are probably the reason why only 32% of cases of AHS were explained by the annual rainfall received. Further research is required to investigate these variables.

For instance, vegetation in the south-western Khomas Region was reported as medium–dense to sparse shrubland (Table 3.1). This means the soil may be mostly exposed to full sun, which is favoured by species like *Culicoides imicola* Kieffer (Conte *et al*., 2007), provided soil moisture and resting sites were available. With February as the highest rainfall month, the south-western Khomas Region is a summer rainfall area (Table 3.1). Depending on the availability of shelter, warm conditions, combined with summer rains, generally favour the development of *Culicoides* larvae (Nevill, 1967).

At most of the surveyed sites, pools and dams were reported to hold water for most of the year (Table 3.1). The presence of *Culicoides* hosts and breeding habitats provided by pools, may account for the exceptions to the general pattern of cases of AHS observed. The presence of these moisture sources, point to the possibility that potential AHSV *Culicoides* vectors can complete their lifecycles in the arid south-western Khomas Region, even during droughts (see section 3.3 for further discussion on *Culicoides* midges.)

If the AHS outbreaks are from an endemic source, the distribution of AHS incidence proportion seen in Fig. 3.4 may be attributed to variation in the water retention capabilities of the sites in the escarpment, rather than the rainfall received initially. This may be either in the form of soil moisture for breeding sites and/or moist air pockets in hollows which serve as resting places for *Culicoides* adults. When comparing rainfall patterns generated in Fig. 3.4, to the topography of the area (Appendix 4), it is observed that there are shared patterns of rainfall and topography; the higher rainfall values recorded were generally on the plateau and along the ridge of the Khomas Hochland Mountains with a sharp decrease in rainfall along the escarpment towards the lowlands. It is proposed that higher areas drain faster than low-lying areas, which may explain why AHS incidence proportion is lower at the highest locations. In the lowest rainfall areas, which are also the dry areas, the high evaporation rates may account for limiting vector occurrence and therefore most of the AHS incidence proportion there. However, along the escarpment (Fig. 3.4), where several fold mountains and rugged terrains may provide moist hollows and pools, more infected vectors may be supported for longer periods. This could account for more frequent occurrence of AHS in these areas, should it be found that more *Culicoides* midges were collected at these sites. In section 3.2 it was investigated whether the potential cycling host, the *E. z. hartmannae*, prefered the escarpment zone to the other zones, which may cause horse populations in the escarpment to be exposed to viral challenges more frequently and at greater intensities.

The horse populations, and individuals within these populations, might not be equally susceptible to AHS, since it was observed that all the horses that had died from AHS in the south-western Khomas Region were of a group in the population which might have compromised or naïve immune systems (such as foals (Table 3.2)). The possibility that there might be individuals within the horse population (that were largely not vaccinated) which were less affected by the disease, suggests the possibility that horses may play a greater role in the reservoir pool than initially expected. This possibility should be investigated in future.

Management of horses and other anthropological activity might also account for only 32% of the AHS incidence proportion being explained by the annual rainfall received and the general distribution of AHS incidence proportion shown in Fig. 3.4. From the questionnaire data (Table 3.1 and Table 3.2), it was observed that only 46% of farmers vaccinate their horses and only 38% do so regularly. The occasional outbreaks of AHS might have been attributed to insufficient number of horses vaccinated over the entire area. Coetzer & Guthrie (2004) reported that at least 80% of the horses in an area need be vaccinated for the vaccine practice to be successful. Many farmers did not consider AHS a threat to their horse stock, as was also reflected by the low vaccination percentage. Others considered the vaccinations ineffective and too costly to justify usage. Since horses were not kept in closed stables, but allowed to roam free, they may be frequently exposed to areas and times at which *Culicoides* midges are most active. Anthropological activity may also modify the environment in favour of *Culicoides* midges. Further research is underway to determine the anthropogenic effect on the number of *Culicoides* midges and community species composition compared with 'naturally' occurring communities which should represent *Culicoides* midge communities in arid regions more accurately.

On the other hand, *Culicoides* midges might be entirely absent and cases of AHS may not be due to a local virus source, but rather a result of faulty vaccinations. If the latter case is true, this may explain the apparent low dependence on mean annual rainfall for the occurrence of AHS. It must therefore be demonstrated that potential AHS *Culicoides* species vectors can be sustained in the south-western Khomas Region as investigated in section 3.3 and discussed in 3.3.4.

In context with the rest of southern Africa (Meiswinkel *et al.,* 2004; Venter *et al.,* 2006), AHS appears to have been relatively infrequent in the south-western Khomas Region for the last five years (Table 3.2) (cases of AHS at some farms in the area were restricted to one or two reported cases over many decades). However, in some areas, large AHS outbreaks appeared to occur on occasion. At a few sites, such as at Hureb Süd and Tsauwasis, high casualties due to AHS seem to be annually recurrent and AHS is considered a significant problem at these sites. Hureb Süd does not appear to be significantly different from the other locations in terms of rainfall – yet cases of AHS were consistently high.

In Fig. 3.5, however, it was shown that periods of high or low rainfall may stretch over seven to ten years, and it is possible that the past five years fall in one of either of the extremes and is therefore not truly representative of the occurrence of AHS in the south-western Khomas Region for its normal climatic range.

In support of this observation, rainfall across the south-western Khomas Region varied both in terms of quantity and distribution (Fig. 3.5 and Appendix 4). In the case of 2009/2010; rainfall deviated below the norm, where in 2010/2011, the area received two to three times the mean annual rainfall.

Compared with the mean annual rainfall pattern and distribution, the rainfall for the year 2009/2010 varied considerably from the average (112 mm below average) (Table 3.3). The *Culicoides* collections for this period can therefore be ruled as the lowest limit occurrence or 'worse-case scenario'. Research is underway to evaluate the occurrence of *Culicoides* midge communities over the 2009/2010 period. The presence of *Culicodes* midges in winter may indicate that occurrence of *Culicoides* midges may increase several fold during periods of average to above average rainfall, as observed in the year 2010/2011 (Table 3.3), where the rainfall exceeded the mean annual rainfall by 626 mm.

It was also important to note that six out of ten sites sampled for 2010/2011 reported cases of AHS in horses compared with two sites out of the fifteen sites sampled for 2009/2010. The incidence proportion also increased from 2009/2010 to 2010/2011 from, on average, 1-7%. An increase in rainfall appeared to be the reason for this increase.

If only dependent on climatic conditions, it is possible that episodic influx of *C. imicola* from the interior to the drier west may occur. A large increase in *Culicoides* population may also result from the sudden increase in numbers from the small populations surviving in ephemeral micro-habitats. Such increases in *Culicoides* midge numbers may cause unexpected AHS outbreaks should AHSV be present in local reservoirs.

Across the landscape in the south-western Khomas Region, and at certain periods, conditions may become favourable in the study area to support ASHV cycling.

3.2. THE HARTMANN'S MOUNTAIN ZEBRA AS A POSSIBLE RESERVOIR OF THE AFRICAN HORSE SICKNESS VIRUS IN THE SOUTH-WESTERN KHOMAS REGION, NAMIBIA

3.2.1. Introduction

To verify that AHS is enzootic to the south-western Khomas Region, the virus source must be indigenous or occur permanently in the area (Higgs & Beaty, 2005), i.e. it is not imported by chance from another enzootic area or introduced by vaccine practices. In the south-western Khomas Region, the Hartmann's mountain zebra (*E. z. hartmannae*) is expected to act as a cycling host for AHS.

It has often been stated that zebras are suspected of serving as cycling host for AHS (Binepal *et al.,* 1992; Lord *et al.,* 1997; Barnard, 1993; 1998). Some research has been conducted on the occurrence of the disease in Burchell's zebra (*Equus burchelli*) (Barnard, 1993; 1994: 1998; Lord *et al.,* 1997), but no information regarding AHS in another zebra species, *E. z. hartmannae* exists. *E. z. hartmannae* is reported to be widespread in the current study area, the south-western Khomas Region (Mendelsohn *et al.,* 2002).

It is yet to be proven that the *E. z. hartmannae* can become infected with the AHSV and demonstrate virions in the blood to confirm its status as a cycling host (Higgs & Beaty, 2005) for review on arbovirus reservoirs). Reservoir hosts must also be present in high enough densities to classify AHS as enzootic to the south-western Khomas Region.

Horses and mules are the animals most afflicted by the disease (Binepal *et al.,* 1992); however they do not appear to be the reservoir hosts, as they die quickly from the disease, and the effective infective duration is short. This should eliminate them from the maintenance role of the virus in the area. They may, however, play a significant role in the amplification or epidemic phase of the viral cycle as is often found in 'accidental' susceptible hosts of arboviruses (Higgs & Beaty, 2005). However, naturalised horses must also be investigated for the possibility that they may have developed some resistance against an AHS infection and their role in the initiation of AHS outbreaks should not be ruled out entirely (see also Weyer, 2010). Yet, considering that horses were not present in this part of Africa in the pre-colonial era (Henning, 1956; Coetzer & Guthrie, 2004), it is expected that the virus must be able to perpetuate in a zebra population alone, albeit at lower total virus counts than when amplification hosts are present.

Other possible long term hosts, such as donkeys and mules are few in number (Mendelsohn *et al*., 2002) and their role as cycling hosts is expected to be negligible. *E. z. hartmannae* is therefore the suspected cycling host to be investigated for the maintenance phase of the AHS viral cycle for this study.

The availability of susceptible hosts is determined by the following: (1) the fluctuation in the population of zebra in the area and (2) the proportion of the cycling host (in this case, zebra) population which demonstrates viremia (Higgs & Beaty, 2005).

3.2.1.1. The occurrence of AHSV in the vertebrate host

The virus infects horses, donkeys, mules and zebras (Binepal *et al.,* 1992; Coetzer & Guthrie, 2004), but of this list, only zebra (more specifically *E. burchelli*) appear to have no clinical symptoms of the disease (Binepal *et al.,* 1992; Barnard, 1993; 1998; Coetzer & Guthrie, 2004), although they are readily infected by the virus (Lubroth, 1988; Rodriguez *et al.,* 1992; Barnard, 1993; 1998). There are nine serotypes of AHSV, all of which are endemic to South Africa although they do not commonly occur in equal abundance (McIntosh, 1958; Howell, 1962). The occurrence of different serotypes in Namibia must still be investigated fully; however Scacchia *et al*. (2009) isolated viruses of serotype 1, 2, 4 and 9 from nine horses north and east of Windhoek. It is expected that, like in South Africa, all nine serotypes should be circulating in Namibia. Immunity against one serotype does not ensure immunity against another and thus, theoretically, a susceptible host may become infected with AHSV nine times during its lifetime.

In arboviral diseases, local viral infection of the muscle and surrounding cells occur (Higgs & Beaty, 2005) after the vector takes a blood meal. Thereafter it manifests in the lymph nodes (Coetzer & Erasmus, 1994; Mellor & Hamblin, 2004). This is evident as primary viremia, which is of a far lower count than the secondary viremic outbreak (Higgs & Beaty, 2005).

Replication of the virus in these tissues and the release into the vascular system enables the tissues in other parts of the body to become infected – such as the spleen, heart, lungs, caecum, pharynx and choroid plexus (Coetzer & Guthrie, 2004), as evident by the high concentration of the virion found in these organs. In horses, a probe test for viral RNA has indicated that in general, the virus very likely replicates in the endothelial cells (and those cells that are morphologically similar) of these organs; and mononuclear cells of the spleen (Brown *et al.,* 1994; Clift & Penrith, 2010).

Organs that are rich in capillaries are most affected (Brown *et al*., 1994; Kuno & Chang, 2005), perhaps because the virus is associated with red blood cells during viremia. The virion enters receptive cells of these organs by means of endocytosis, losing its outer capsule in the process

(Matsuo *et al.,* 2010). Replication of the virus then occurs in these organs and thereafter, the virion is again released into the bloodstream – during the secondary viremia (Higgs & Beaty, 2005).

Replication that occurs in the vascular system produces a high titred virus count at $Log_{10}5.0$ $TCID_{50}$/ml in horses and lasts four to eight days (Coetzer & Guthrie, 2004). At this time, virions are available to be ingested by a vector during a blood meal. It is at this time that bite to infection ratio is suspected to be high and the proportion of midges infected large. This likewise increases host infection per *Culicoides* midge bite (Higgs & Beaty, 2005).

After the secondary viremic episode – and if the animal(s) survived, viable viruses are no longer detectable in the blood of hosts by virus isolation (Mellor, 1993) and the infection of *Culicoides* midges by AHSV during blood-feeding in the following season is therefore unknown. In an immune host with a matured immune system, a virus challenge is not likely detected by virus isolation.

Where there was an infection in the past, it can be detected by testing for antibodies against AHSV. In *E. burchelli*, AHS infection was demonstrated by the presence of antibodies against AHSV (Hamblin *et al.,* 1992; Binepal *et al.,* 1992; Barnard, 1993). Similarly, AHS antibodies must be isolated from *E. z. hartmannae* to prove that this zebra species is also infected with AHS and that there is an endemic viral source in the area. However, the presence of antibodies will also indicate the likely cessation of viremia due to a successful immune response against the virus challenge. Therefore if antibodies are detected in a particular individual, then virus isolation is unlikely to yield any positive results.

Under laboratory conditions, Barnard *et al.* (1994) (see also Wilson *et al.,* 2009) it was found that it is possible to isolate AHSV for 40 days in *E. burchelli* blood, and in organs such as the spleen, for up to 48 days post virus inoculation. Therefore viral isolation in the field could indicate a 'recent' infection within the 40 to 48 day time frame. Since the virus is usually isolated in the blood during the febrile (or viremic) stage (Mellor, 1993), it is possible that zebras can be infective for that period. However, a low viremia virus titre was demonstrated, at less than $Log_{10}3.0$ $TCID_{50}$/ml (Coetzer & Erasmus, 1994). It may yet be possible that zebra are only tangential hosts. If viremia is too low, their actual contribution to the viral cycle may be less significant than originally suspected, although the outbreaks in Spain seem to point to the essential presence of zebra to enable recurrent outbreaks (Lubroth, 1988; Rodriguez *et al.,* 1992).

Weyer (2010) detected viral RNA in clinical AHS field infected horses for a considerably longer period than the 21-day period virus isolation tests have indicated thus far (Coetzer & Guthrie, 2004). However, whether or not the presence of viral RNA indicated viremia, is still uncertain. It is expected that with viral RNA detection (Weyer, 2010), AHS infected zebra may also show a possible extended infective period, beyond the current established 40 days, with implications for zebra and their role as reservoirs.

3.2.1.2. The migration habits of Hartmann's mountain zebra (*E. z. hartmannae*) in the south-western Khomas Region

E. z. hartmannae as reported in Cillié (2004) is a different species to the Burchell's zebra (*E. burchelli*) tested in AHS epidemiology studies thus far. In the plains it is believed that *E. burchelli* serve as the cycling host for AHS, however, since they do not roam the mountains of the south-western Khomas Region (Mendelsohn *et al.,* 2002), this did not account for any cases of the disease in this area.

E. z. hartmannae is physiologically different from their plains-dwelling counterparts: with a larger heart (0.97 kg more on average) and hooves that grow at a faster rate (Joubert, 1973). The two zebra species are also divided geographically, since *E. z. hartmannae* prefers highlands, slopes, rugged and mountainous terrain (Joubert, 1973), whereas *E. burchelli* is better adapted to flat, open plains. There is only a small area where their ranges overlap, according to Mendelsohn *et al.* (2002). Joubert (1973) proposed that *E. z. hartmannae* preference for such terrain is due to the availability of rock pools within the valleys, compared with more limited availability in gentler topographies. The plants preferred by *E. z. hartmannae* are also to be found in the vegetation type occurring only in the escarpment. The rugged mountains may also provide the zebra refuge from predation, since some predators (like man) are less suited to traversing the mountainous landscape. AHS is not normally associated with mountainous terrain, since zebra do not normally inhabit these landscapes. However, since *E. z. hartmannae* favour mountainous areas, this points to the possibility of the natural cycling of AHS.

It was observed by Joubert (1973), that despite intensive habitat encroachment and hunting of *E. z. hartmannae* in the Khomas Hochland highlands, the animals appeared to occur in their highest densities in this area, with their numbers tapering off gradually to the north and to the south. At present, however, it is uncertain how many *E. z. hartmannae* actually occur on the escarpments of the Khomas Region, but local opinion holds that they are still relatively abundant.

The current counts of *E. z. hartmannae* population at the nearby Namib Naukluft Park, number approximately 3 502 animals over an area of about 50 000 km^2 and is approximately estimated to occur at density of about 0.07 zebra/km^2, according to the most recently published census by the Ministery of Environment and Tourism (MET) survey in the year 2000. Local people believe the zebra trek from the Namib Naukluft Park via the Gaub and Kuiseb river valleys into the farming areas. It is estimated that there are about 16 400 *E. z. hartmannae* in Namibia in total.

The vector and host must be in the same place at the same time to allow for effective viral cycling to occur (Higgs & Beaty, 2005). Since *E. z. hartmannae* migrate along the river valleys to utilise the pools from remnant ephemeral rivers during the dry months, and the vector also requires moist soils to prevail for more than seven days (Meiswinkel & Paweska, 2003), opportunities may arise for *Culicoides* midges to take a blood meal of *E. z. hartmannae* and successfully transfer the AHSV. It should also be considered that *E. z. hartmannae* is highly mobile (Cillié, 2004). This is significant because even if arid conditions isolate *Culicoides* midge breeding sites and limit host numbers, isolated, non-infected *Culicoides* populations can possibly be exposed to AHSV in hosts and *vice versa*.

The availability of weaned foals was also of importance, as it is believed that it is during this time period during which zebra are most susceptible to the virus, as they were no longer protected by passive immunity (Barnard, 1993). It is proposed that the virus was maintained by the circulation of AHSV between weaned foals and *Culicoides* vectors present throughout the year. As mentioned previously, a naive host can theoretically be infected nine times by different serotypes of AHSV, with a viremia which may last for about 40 days per infection (Barnard *et al.,* 1994), which suggested a very long potential duration of viremia in weaned zebra foals.

It is therefore the objective of this part of the study to determine whether or not the *E. z. hartmannae* showed signs of AHS infection in blood and tissue samples, and is able to act as cycling host or form part of the reservoir pool in the south-western Khomas Region.

It will also be investigated in this study whether or not *E. z. hartmannae* was abundant enough in the south-western Khomas Region and/or whether or not their home ranges extended into the area. Their migration habits will therefore also be addressed in the survey, as well as the potential availability of susceptible weaned foals.

3.2.2. Materials and methods

3.2.2.1. The study area

See Chapter 2.1.

3.2.2.2. *E. z. hartmannae* migration based upon local farmers' opinion

The same farmers surveyed in Table 3.1 were questioned in the same manner as described in paragraph 3.1.2.2 on the habits of *E. z. hartmannae* in the area. The questions in the questionnaire applicable to the migration habits of *E. z. hartmannae* were:

(i) What is the estimated number of Hartmann's mountain zebra (*E. z. hartmannae*) on the farm?

(ii) Zebra foaling period: is it restricted to one season or does it occur throughout the year?

(iii) Hartmann's mountain zebra (*E. z. hartmannae)* home range extent and migration patterns:
  - If they migrate, where do they go?
  - Do they move away or towards the farm during periods of drought?

(iv) The use of zebra (tourism, professional hunting, food).

The responses were tabled and the information was used to compile maps for spatial analysis of the zebra distributions in conjunction with the environmental variables summarised in 3.1.3.1 and discussed under 3.1.4.

3.2.2.3. Blood and Tissue sampling procedure from *E. z. hartmannae*

Co-operation with land owners who held permits for zebra hunting was obtained for the collection of Hartmann`s mountain zebra blood and tissue samples. The animals sampled were those shot for the professional hunting industry or for meat supply for farm-labourers. The disadvantage of this method is the non-randomness of the selection. Animals shot for trophies were expected to be adult stallions due to hunter's bias. The selection of animals for meat supply may be less selective for gender, but may still show a selection bias towards adult zebras. The likelihood for virus isolation from zebras of this demographic is low.

The zebras were sampled for blood and either spleen, lung or liver tissues. A kit was provided for the farmers and game-hunting reserve owners in the study area for the collection of these samples (Fig. 3.6). In the kit the following information and question sheets were included: a question sheet (Fig. 3.7), an instruction sheet for the sampling and handling of the zebra blood and tissue samples (Fig. 3.8). All the sample containers in the same kit were marked with the same number to denote that they all came from one specific zebra. The question sheet (Fig. 3.7) was assigned the same number (for example 001) as all the sample containers in a kit. This linked the blood samples to the location and the person who had taken the sample. Each container was labelled and denoted with code 'A' – blood serum; 'B' and 'C' – whole blood; 'D' and 'E'– spleen and/or lung tissue sample. Two-hundred sampling kits were distributed amongst the farmers. Vacutainers® were provided for blood collection: Two Lavender/Purple Tops with EDTA for whole-blood samples and Gold Tops SST^{TM} for serum samples. Urine sample bottles were provided for tissue samples.

Sampling should require the least possible effort, while retaining quality for accurate results. It was advised that the samples should be kept cool and placed in a refrigerator at 4°C until the time of collection and analysis. However, the very remote and rugged conditions of the survey area, consequently, the inability to predict when the opportunity for sampling would presents itself, allowed for inadequate preparation. Farmers report that often coolants were not at hand for sampling opportunities, which occurred unexpectedly. The samples were therefore not always collected under the best conditions.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) – and Enzyme-linked Immunosorbent Assay (ELISA) tests were conducted on the blood and tissue samples by the Namibian Central Veterinarian Laboratory in Windhoek as discussed in 3.2.2.4 below. Samples were taken at irregular intervals from July 2009 to the end of 2010.

Figure 3.6. Zebra blood and tissue collection kit provided to the farmers with instruction and question sheets.

Sampling blood, urine and spleen tissue from zebra carcasses

Attribute information sheet

Farm name: ______________________

Name of sample-collector: ______________________

Date: ______________________

GPS Co-ordinates (if known) ______________________ South
______________________ East

Concerning the zebra:

Male ☐ Female ☐
Approx. age ______________________

Young adult ☐
Adult ☐
Elderly ☐

Please check the appropriate boxes regarding physical condition of the zebra:

Pregnant ☐
Eye infection ☐
Abscesses, injuries ☐
Bumps/bulges on stomach ☐
Heavy worm infestation of intestines ☐
Excessive mucus visible in nostrils ☐

Any other observations or comments: ______________________
____
____

Figure 3.7. An example of the question sheet to be completed by the hunter to accompany the blood and tissue sample kit shown in Fig. 3.6.

Sampling of Zebra Carcasses for Blood, Spleen and Urine

Contact **Elbè Becker** at:
Cell (SA): **0027 83 980 9061**
Cell (Nam): **081 433 5710**
Email: **ahs.zebra@gmail.com**

Inventory

Sampling equipment is labelled by the following numbers:

1. Gold Top Vaccutainer® (Yellow vial)
2. Purple Top Vaccutainer® (Purple vial)
3. Syringe
4. 18G needle
5. Spleen (milt) -sample bottle
6. Urine bottle
7. Disposal bag
8. Gloves
9. Checklist

Sampling: Blood and Spleen tissue taken from dead zebra

After the zebra is shot, time is of the essence

1. Affix the 18G needle to the syringe, leave protective cap on.
2. Put gloves on
3. Keep the Gold Top and Purple Top vacutainers at hand
4. Remove the protective cap from the 18G needle
5. Extract blood from the jugular vein (slagaar) using the syringe (see next page)
6. Inject the blood into the Gold Top $^{\text{TM}}$ and Purple Top$^{\text{TM}}$ vacutainers
7. Complete the checklist

(The following steps can be conducted when and where the animal is slaughtered)

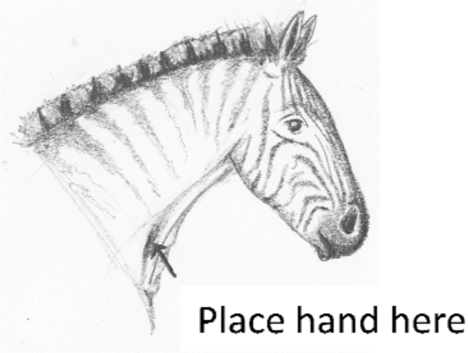
8. Cut a sample of the spleen/lung/heart and place it in the spleen-sample bottle
9. Place all samples and checklist into a Ziploc bag. Replace the pink cap of the needle – put used holder, needles, syringe and gloves into the disposal bag.
10. Place the Ziploc bag in a cooler box for transportation
11. Please refrigerate the sample bag as soon as possible – **do not freeze**

Please inform me (see details above) that the samples had been taken as soon as possible. Collection thereof will then be organised.

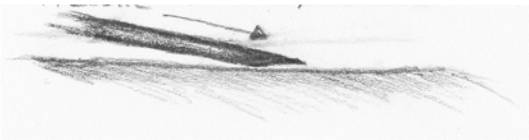
Figure 3.8. Zebra blood and tissue collection procedure leaflet accompanying the blood and tissue sampling kit shown in Fig. 3.6.

Technique for drawing blood from a zebra carcass:

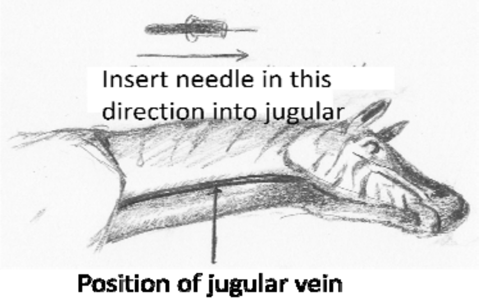
1. Turn the animal's head to the natural position to make the jugular vein more visible
2. Place left hand on indentation of lower part of the zebra's neck, ¾ of the way down towards the chest



3. Jugular vein (*nekslagaar*) fills with blood and one can see the vein
4. Extract blood using the syringe.
5. The needle must be held at a slight angle – flat tip facing down as shown below



6. Insert the needle in the direction of the zebra's head
7. As the hide is tough, the needle may need more force to pierce the hide effectively – a quick single stabbing action is necessary
8. Extract as much blood as possible



Thank you very much for your kind help.

Figure 3.8. (continued) Zebra blood and tissue collection procedure leaflet to accompanying the blood and tissue sampling kit shown in Fig. 3.6.

3.2.2.4. Laboratory analysis of *E. z. hartmannae* blood and tissue samples

Zebra blood and tissue samples were tested by the Central Veterinary Laboratory in Windhoek for anti-AHSV antibodies by the use of Enzyme-linked Immunosorbent Assays (ELISA's) (Hamblin *et al*., 1991; Maree & Paweska, 2005) and AHSV RNA by RT-PCR analysis (Stone-Marschat *et al.,* 1994; Quan *et al.,* 2010).

The ELISA testing used for the analysis of blood serum was done as described by Hamblin *et al.* (1991). The test did not differentiate between serotypes, and therefore it tested only for absence/presence of antibodies. The analysis will not indicate whether or not AHS infections have occurred over the recent long term as the test acts on Immunoglobulin G (IgG) (Maree & Paweska, 2005), which can remain in the animal's system long after the initial challenge.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) analysis was done of the whole-blood samples as described by Stone-Marschat *et al.* (1994) for the detection of viral RNA. If the samples tested were positive, a reserve sample was sent to Italy at the *Instituto Zooprofilattico Sperimentale dell' Abruzzo e del Molise 'G. Caporale*, where viral isolation and serotyping was conducted (see also Coetzer & Guthrie, 2004).

Virus isolation is made by the inoculation of cell cultures (Sailleau *et al*., 1997). Viral isolation was used to indicate a current infection. The failure to isolate viable viruses may have produced a false-negative as the test is sensitive to the quality of the samples (see paragraph 3.2.2.3).

To determine whether or not the virus reached the endothelium of organs usually associated with its replication (Brown *et al*., 1994; Kuno & Chang, 2005), tissues such as spleen and lungs were tested for viral material by Polymerase Chain Reaction (RT-PCR) analysis.

3.2.2.5. Data analysis

(a) Spatial analysis of Hartmann`s mountain zebra (*E. z. hartmannae*) questionnaire data

To illustrate patterns of occurrence and distribution of zebra in context with certain environmental variables and the occurrence of AHS, ArcGIS® software was used to map and interpolate the data as discussed in 3.1.2.4(c).

The data collection of *E. z. hartmannae* numbers were recorded as point data, where in reality, the farmer represented the value as observed over a larger area. The original point data was patchy, but the Inverse Distance Weighting algorithm provided in the ArcGIS® Spatial Analyst software (Wescott & Brandon, 1999), was used to build a raster to predict the occurrence of *E. z. hartmannae* over the entire study area based on the scattered data points.

The occurrence of AHS incidence proportion was represented as point data expressed as bar graphs representing zebra numbers at each sample point. See also 3.1.2.4(c).

(b) Interpretation of *E. z. hartmannae* blood and tissue analysis

The presence of anti-AHSV antibodies in zebra blood was interpreted as confirmation that the *E. z. hartmannae* can be infected with AHS (Hamblin *et al.,* 1992; Barnard, 1993; Maree & Paweska, 2005). This method detects Immunoglobulin G (IgG) and does not differentiate between a current or latent immune response.

If the virus is isolated from a zebra blood sample, the result will be interpreted as an indication that the animal showed viremia– and should a *Culicoides* midge have taken a blood meal from the animal, the midge could have become infected with AHSV. Such a zebra would therefore be classified as a 'reservoir unit' as part of a possible reservoir pool (Higgs & Beaty, 2005). A positive result could indicate an infection as recent as 40 to 48 days before the sample was taken (Barnard *et al.,* 1994).

Since virus isolation techniques are susceptible to false negatives due to sample quality, the RT-PCR analysis of blood and tissue with positive results may be used to suggest viremia in *E. z. hartmannae*, since it is a more sensitive test (Quan *et al.,* 2010; Weyer, 2010), although it must still be proven that the presence of viral RNA indicates a current viremic event.

3.2.3. Results

3.2.3.1. The migration habits of Hartmann's mountain zebra (*E. z. hartmannae*) in the south-western Khomas Region: questionnaire data

Table 3.6 below displays a summary of data provided telephonically by farmers in the south-western Khomas Region. Fig. 3.9 and Fig. 3.10 relate the zebra data provided by the farmers with the environmental data collected in 3.1.2.2 and discussed in 3.1.4. Fig. 3.9 shows the distribution of zebra during the wet season, and Fig. 3.10 shows the distribution of zebra during the dry season. This shows where the potential cycling host is found at the maintenance or amplification phase for AHS – and at the time when *Culicoides* midge numbers are expected to be at their peak. See Appendix 4 for additional graphical presentations of the occurrence of *E. z. hartmannae* in relation to the various environmental data obtained in the questionnaire as summarised under 3.1.3.1.

The data presented in Table 3.6 represent farmers' perceptions and estimates. Therefore the data is subject to individual bias. The data points were also not equally distributed over the area and are patchy in nature. The data therefore indicates trends, rather than precise data.

According to expert opinion (farmers), *E. z. hartmannae* foal throughout the year.

In general, the highest numbers of *E. z. hartmannae* were found in the south-western corner of the study area (Fig. 3.9), with an isolated area of high numbers in the north-east at Claratal. At Tsauwasis, Groot Hakos and Isabis, there were apparent anomalies with very low zebra numbers during a wet period compared with the predicted value of their surrounding areas.

During high rainfall periods, highest concentrations of zebra were found at lower rainfall zones (Fig. 3.9), which was also incidentally at the lower altitudes, or pediment. During drier periods, *E. z. hartmannae* numbers increased in the area, especially towards the north-east (Fig. 3.10). Their range extended slightly into higher altitudes (Appendix 4), but they avoided the highest areas of the Khomas Hochland. Further south, they ventured onto higher altitudes.

The difference between the wet and dry period zebra numbers (Fig. 3.9 and Fig. 3.10) seemed to be greatest at higher altitudes. On the pediment, at sites such as Corona and Swartkrans, there was no apparent difference between wet and dry period zebra numbers.

The occurrence of highest AHS incidence proportion (Fig. 3.9 and Fig. 3.10) was located largely in the north-west, where zebra were present but were fewer in number than in the south-east, where AHS incidence proportion was very low or entirely absent. At Tsauwasis there was a relatively high number of cases of AHS, yet there were very few zebra present.

Table 3.6. Expert opinion on the occurrence of Hartmann's mountain zebra (*E. z. hartmannae*) as determined by a questionnaire conducted in March 2009
(Refer also to paragraph 3.1.2.2)

Farm Name	**Estimated zebra no.**		**Zebra immigration to local area**	**Zebra births**
	Min	**Max**		
Hochfels-airy	60	70	Present all year	All year
Aandrus	10	12	Dry season	Unknown
Neu Heusis	30	50	Dry season	Most Sept -Dec
Heusis	5	15	Dry season	All year
Groenkloof	50	50	Dry season	All year
Hureb Süd	60	200	Dry season	All year
Tsauwasis	15	60	Dry season	All year
Harmonie	200	250	Dry season	All year
Jonkersgrab	100	200	Present all year	All year
Wasserfall Valei	100	200	Dry season	All year
Farm Hochland	90	300	Unknown	All year
Kobos	100	120	Dry season	Seasonal
Amor	200	500	Dry season	All year
Landmeister	15	90	Present all year	All year
Kiamsab	200	200+	Unknown	All year
Weissenfels	200	300	Most in dry	Dry season
Chaibis	300	500	Most in dry season	All year
Corona	600	600	Dry season	All year
Swartkrans	800	850	Dry season	All year
Natas	350	600	Dry season	All year
Isabis	0	10	Present all year	Unknown
Hakos	2	400	Dry season	All year
Weenen	200	380	Dry season	All year
Claratal	250	600	Wet season	All year

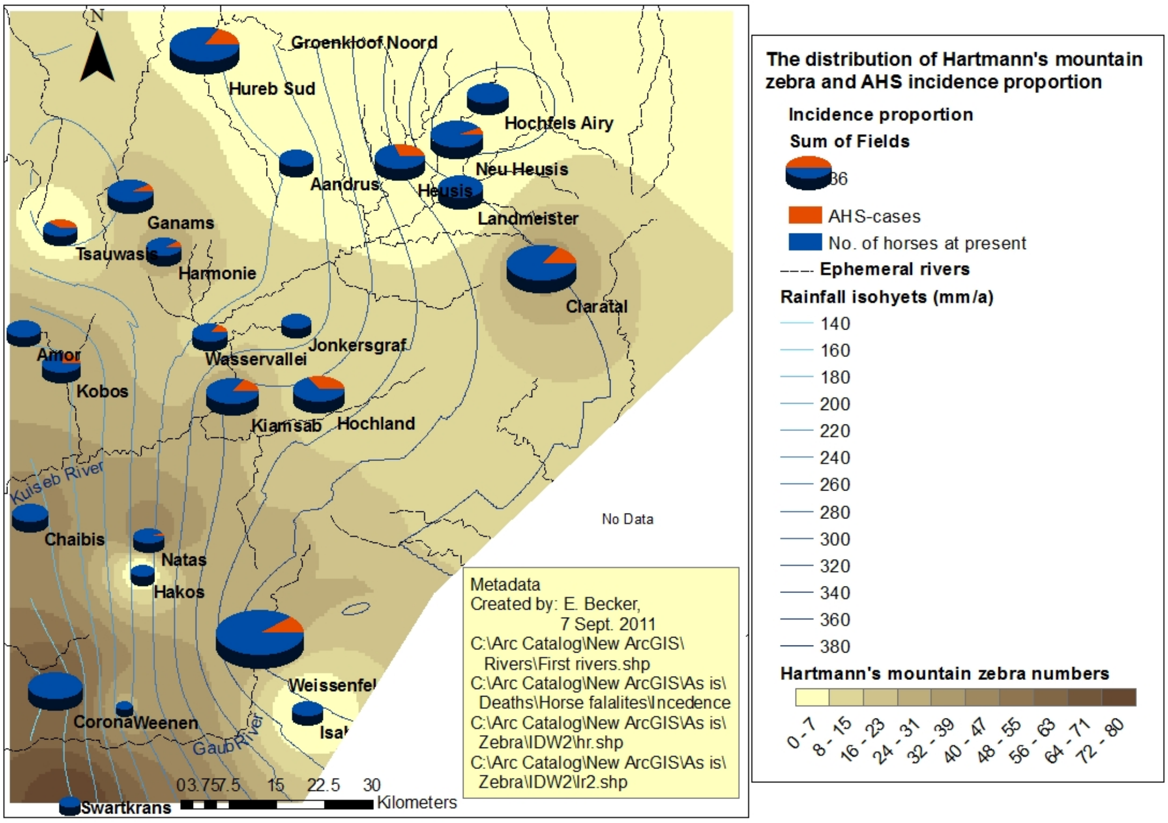


Figure 3.9. *E. z. hartmannae* occurrence during estimated high rainfall periods and the AHS incidence proportion in the south-western Khomas Region from 2004 to 2009.
Refer to Table 3.1, Table 3.2 and Table 3.3 for input data.

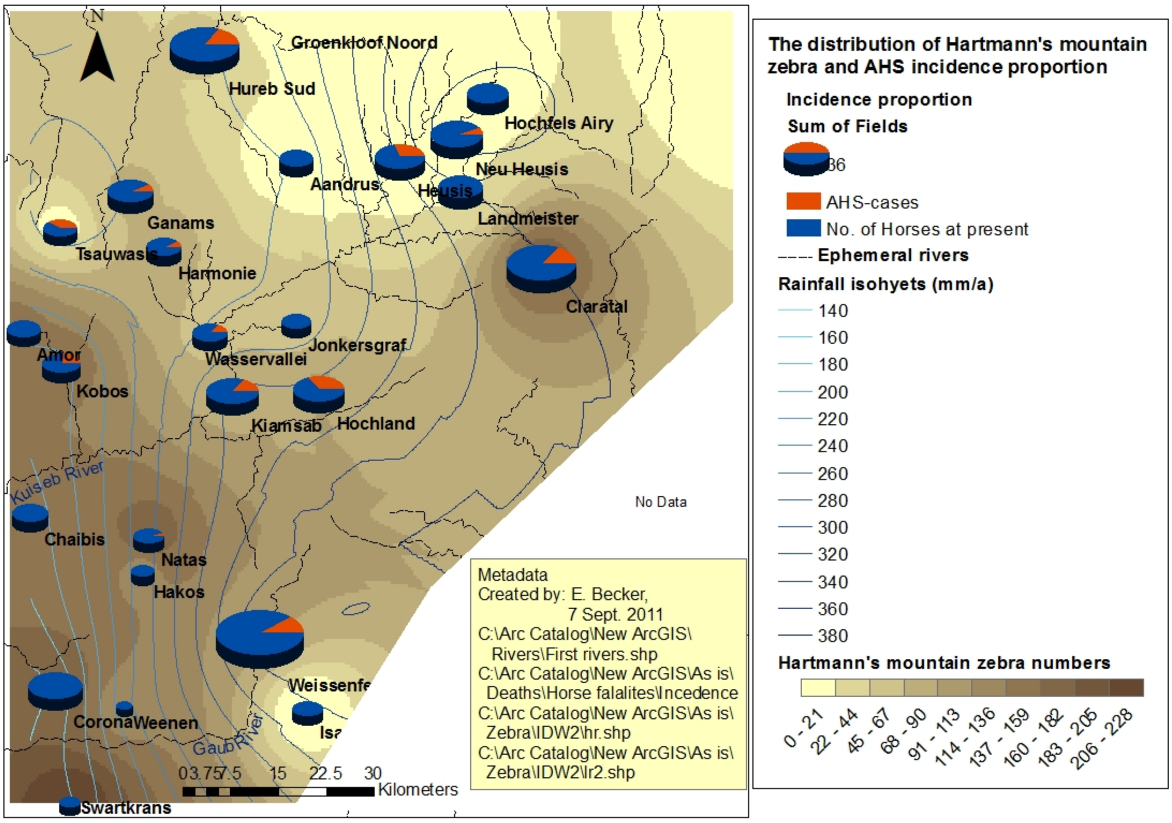


Figure 3.10. *E. z. hartmannae* occurrence during estimated low rainfall periods and the AHS incidence proportion in the south-western Khomas Region from 2004 to 2009.

Refer to Table 3.1, Table 3.2 and Table 3.6 for input data.

3.2.3.2. The occurrence of AHSV in *E. z. hartmannae* blood and tissue samples

Table 3.7 displays a summary of the zebra blood and tissue samples collected by hunters and farmers were analysed by the Central Veterinary Laboratory in Windhoek. The samples were taken sporadically during 2009 and 2010. The table also summarised the additional information about the individual zebras sampled as recorded on the sampling kit question sheet by the hunter (see 3.2.2.3).

Not all the question sheets accompanying the kits had been fully completed, so for purposes of describing the zebra population from the sample, only the completed samples were considered. Not all of the sampled zebra were sampled for tissues such as the spleen or the lung. The samples were mostly taken when rainfall values and temperatures were low as this was the main hunting season.

Table 3.7. *E. z. hartmannae* individuals sampled attribute information and ELISA, RT-PCR and viral isolation test results of their tissue and blood samples taken in the south-western Khomas Region, taken during 2009 to 2010.

Sample	**Date**	**Gender**	**Age**	**Disease /injury**	**ELISA**	**RT-PCR (Blood)**	**RT-PCR (Tissue)**	**Viral isolation**
_001	07/07/2009	Male	Young adult	No	+	-	-	n.d
_002	17/07/2009	Male	Elderly	No	+	-	+	n.d
_003	03/07/2009	Male	Elderly	No	+	+	+	-
_004	13/07/2009	Male	Adult	No	+	-	+	n.d
_005	19/07/2009	n.d.	n.d.	No	+	-	-	n.d
_006	n.d	n.d	n.d.	n.d	+	-	-	n.d
_007	n.d	n.d	n.d.	Yes	+	-	-	n.d
_008	10/08/2009	n.d	n.d.	No	+	+	-	-
_009	10/08/2009	n.d.	n.d.	No	+	-	-	n.d
_010	27/07/2009	n.d	n.d.	Yes	+	-	-	n.d
_011	n.d	n.d	n.d	n.d	+	-	-	n.d
009B	30/08/2009	Male	Young adult	No	+	-	-	n.d
_012	n.d	n.d	n.d	n.d	+	-	-	n.d
_013	17/07/2009	Male	Young adult	Yes	+	+	-	-
_014	n.d	n.d	n.d	n.d	+	-	-	n.d
031b	n.d	n.d.	n.d.	n.d.	+	-	-	n.d
032b	29/06/2010	Male	Young adult	No	n.d	+	+	-
160b	29/06/2010	Female	Young adult	No	n.d	+	+	-
_015	15/01/2010	n.d.	n.d.	No	n.d	-	-	n.d
_016	19/01/2010	n.d.	n.d.	No	n.d	-	-	n.d
048b	05/10/2010	Female	9-14 months	No	n.d	-	-	n.d
051b	13/09/2010	Male	Young adult	No	n.d	-	-	n.d
056b	13/06/2010	Male	Young adult	No	+	-	-	n.d
058b	17/05/2010	Female	Adult	No	n.d	-	-	n.d
059b	05/10/2010	Female	Young adult	No	n.d	-	-	n.d
060b	19/11/2010	Male	Young adult	Yes	+	-	-	n.d
061b	17/05/2010	Female	Adult	Yes	n.d	-	-	n.d
066b	29/09/2010	Male	8 months	Yes	n.d	-	-	n.d
067b	17/05/2010	Male	Adult	No	n.d	+	-	n.d
070b	10/06/2010	Female	Young adult	No	n.d	-	-	n.d
071b	13/09/2010	Male	Young adult	No	n.d	-	-	n.d

+ Positive - Negative n.d. No data
Young adult: 2-10 years; Adult: 10-15 years; Elderly: 15+ years

Of the 23 samples, 20 were taken during the winter months (June to September) and three in the warm, dry month of November (Table 3.7).

Nineteen samples' answer sheets identified gender, of which 13/19 (68%) animals shot were stallions (Table 3.7). Mares comprised 6/19 at 31%. In terms of age structure, 2/19 (11%) were foals, one about eight months old and another between nine to 14 months old. Eleven out of 19 (58%) were young adults, 4/19 (21%) were adults and 2/19 (11%) were elderly individuals.

All of the useable serum samples tested for antibodies which indicate the presence of AHSV were found to be positive (Table 3.7). Eight out of 31 (19%) blood samples produced positive results for viral RNA and 8/31 (26%) displayed positive viral RNA in either the blood or tissue. All of these positive samples were taken over the cold, dry months (June to September).

Five out of 31 (16%) of the spleen and lung tissue samples showed a positive presence of viral RNA (Table 3.7), where two of these showed no corresponding presence of viral RNA in the blood. Conversely, of the six viral RNA-positive blood samples, three corresponding tissue samples did not display a positive presence of viral RNA – that is 10% of the zebras sampled. Viable viruses could not be isolated from any of the samples that tested positive for viral RNA.

Four out of 13 (31%) stallions tested positive for viral RNA and 1/6 (17%) mares tested positive for viral RNA – two of the positive samples had no information with regard to gender. Of all the samples that tested positive for viral RNA, 1/8 had no gender record; 3/8 were young adults (2-10 years); 2/8 were adults (10-15 years) and 2/8 were elderly animals. Neither of the two foals (mares) demonstrated positive results for the presence of viral RNA.

3.2.4. Discussion

The results based on the questionnaire data Fig. 3.9 and Fig. 3.10 were based on subjective interpretations – or at least the impressions farmers had of *E. z. hartmannae* on their farms, and standards of estimation may differ among respondents. Fig. 3.9 and Fig. 3.10 therefore illustrate trends rather than precise values. The farms were also selected along the line on the basis that they are contactable by telephone, and was therefore non-random, although relatively unbiased in terms of *E. z. hartmannae* data. As a result, however, the sampled sites were unevenly distributed along the study transect (Fig. 2.2). No other method of obtaining the *E. z. hartmannae* numbers was found to be feasible, nor necessarily more accurate. But these factors may account for the apparent anomalies observed within the interpolated zebra distribution area layer. However, since *E. z. hartmannae* had been present on Isabis farm in the past, the farmer attributed the low number of zebra there to heavy hunting activities on a neighbouring farm.

From the questionnaire (Table 3.6) it is observed that almost all respondents stated that *E. z. hartmannae* foal throughout the year. This implied that there were hosts susceptible to AHSV present all year in the area.

It appears that the *E. z. hartmannae* favour the lower altitudes in the south-west, which are also the lower rainfall zones (Appendix 4). However, when combined with Fig. 3.10, which represents the distribution of zebra during drier conditions, it shows that the zebra moved towards the higher ground and the north-east, areas which receive higher mean annual rainfall. This may indicate that the south-west is the source area. Local farmers report that *E. z. hartmannae* migrated along the Kuiseb and Gaub Rivers and indeed Fig. 3.9 does reveal a slightly higher concentration of zebra along the Kuiseb River and tributaries. However, the distribution of the data points may be too sparsely distributed to show such detail. It is assumed that the movement is driven by the search for grazing and animals must disperse further to reach suitable grazing during the dry period (Fig. 3.10).

This is contrary to the description by Joubert (1973) of the distribution of zebra, where it is reported that the highest concentration of zebra was located on the Khomas Hochland headland. In this study, it appears that zebra numbers increase towards the south-west, towards the pediment. It is possible that a shift of preference of habitat may have occurred over time due to anthropogenic pressure. In support of this claim, stark contrasts were reported at Tsauwasis, Groot Hakos and Isabis, where very low zebra numbers were reported during a wet period compared with their surrounding areas. Isabis reports that in the past, *E. z. hartmannae* were numerous on that farm, but this is no longer the case. For this reason, and from the information provided by Joubert (1973), it is speculated that these locations should have supported similar zebra numbers to their surroundings under normal circumstances. However, due to the current activities of man, they may avoid these sites at present. Perhaps the ‘source’ area for zebra was once more extended along the Kuiseb River up to Claratal, but human activity may since have caused *E. z. hartmannae* to only pass through these areas, rather than remain there permanently.

The distribution of zebra was affected by climatic conditions. During times of high rainfall when the vector is expected to be most numerous, the reservoirs, E. z. hartmannae, were at their lowest numbers, preferring the safety of the lower human inhabitation densities in the south-west.

At Tsauwasis, there were very few zebras (Fig. 3.9 and Fig. 3.10), yet many cases of AHS (Table 3.1). It was apparent, however that there was always a baseline of animals left distributed in the area and only in very few cases do all the animals leave a farm entirely. The question remains, whether or not those left on the farms during the AHS favourable wet periods are sufficient to cause AHS outbreaks.

During the dry periods, they seem to move into areas more affected by human activity. By this means, they may come into contact with midge breeding islands and therefore the influx of zebra in the dry period may make up for the decrease in vector numbers at that time and therefore still permit a low level of AHSV transfer during the dry season.

In general, the recorded higher AHS incidence proportion values do not correspond with the area of highest zebra numbers. However, more objective and replicated data is needed in future research to validate these findings.

The *E. z. hartmannae* sampled for blood and tissue may not represent the *E. z. hartmannae* population in the south-western Khomas Region population very well, since the selection process was not unbiased. Hunters tend to select the largest animals, which are usually the mature stallions. This could account for their 68% representation (Table 3.7.). However, according to Joubert (1972), a typical family generally has more females than males (1:2.2) - except in a bachelor group. The total population male to female ratio in *E. z. hartmannae* was found to be about 1:1, based on foetal gender ratios (Joubert, 1972). The predominance of individuals in the young adult group within a family group is congruous with findings by Joubert (1972). Of all the samples that tested positive for viral RNA, 1/6 had no gender record. The majority of the cases were found in young adults (2-10 years) comprising half the samples which tested positive for AHSV RNA. Only one of these animals was of the adult age group (10-15 years) and 1/6 were elderly animals. It is proposed that once the animals reach a certain age, they may be less susceptible to AHS infections – perhaps due to some developed resistance due to exposure. However, neither of the foals, (which were both mares) demonstrated positive results for the presence of viral RNA. The sample of foals was very small, and although they could have been between eight and 14 months old (and were expected to be weaned (Joubert, 1974)), it may yet be possible that these foals obtained their antibodies through mother's milk. It is unfortunate that the sera were not suitable for antibody testing of these foals. It must be considered, however, that the age determinations were estimates based on interpretations by hunters.

The *E. z. hartmannae* sample size was too small and was not a perfect representation of the zebra population. To draw any conclusions as to the occurrence of AHSV within different demographic groupings and further research in the virology of AHSV in the study area is needed.

The presence of viral RNA in 19 to 26% of the zebra sampled may indicate recent infections i.e. within the last three to four months (see also Weyer, 2010) in these animals, even during an expected 'low' viral circulation phase and among samples taken predominantly in winter. This percentage is expected to be higher during times favourable for *Culicoides* midges.

The samples which tested positive for viral RNA in tissues such as the lung and spleen, but did not test positive for RNA in the blood, is perhaps indicative of a latent infection or perhaps a replication phase of the virus in these tissues (Brown *et al.*, 1994) before the onset of viremia.

Whereas the presence of viral RNA in the blood may be an indication of a viremic event within these individuals, which was 19% of the individuals tested. Some blood samples tested positive for viral RNA, while the corresponding tissue samples did not show a positive presence of viral RNA. These may be individuals demonstrating a primary viremic event, where the virus had not reached and infected the endothelium of the capillary-rich organs from which secondary viremia is launched (Brown *et al*., 1994; Kuno & Chang, 2005). The 10% samples that displayed the presence of viral RNA in both the blood and tissues may indicate that secondary viremia can occur during viral infections of *E. z. hartmannae*.

However, if the virus is capable only of an abortive infection of zebra cells as occurs in some arboviral tangential hosts (Higgs & Beaty, 2005), viral genetic material can still be detected as the zebra cells may permit viral entry, but block full viral replication. In such a case, the detection of viral RNA therefore does not prove that the zebra is a major role player in the maintenance phase of the viral cycle.

Viral isolation from zebra blood is therefore essential, since this is the body fluid ingested by the vector and the source from which it must be infected. If viable viruses (i.e. virions) are not present in the blood and the peripheral blood vessels, the vector cannot acquire the viable virus, even if the virus is capable of inhabiting and replicating in an organ of a host (Kuno & Chang, 2005: Higgs & Beaty, 2005). Further research is needed on how the presence of AHS viral RNA is to be interpreted in terms of indicating infectivity – especially in zebra.

No viable viruses were isolated from the samples and since all of the useable samples tested positive for AHS antibodies, this result is not surprising. Therefore it cannot without doubt be confirmed that *E. z. hartmannae* serve as primary reservoirs for AHSV in the south-western Khomas Region. The absence of viable viruses may be a false-negative, due to the quality of the samples and the difficult conditions under which the samples were collected. Further research must be done to see if viable viruses can be isolated from *E. z. hartmannae* in the field, and which serotypes are present within the population.

The samples were mostly taken when rainfall totals were low to non-existent and temperatures were lower on average. Of the 23 samples, 20 were taken during the winter months (June to September). *Culicoides* midge activity was expected to be very low to zero over these months (see section 3.3), which makes the 100% positive antibody tests surprising.

Since all the useable samples tested for antibodies against AHSV were found to be positive, it is concluded that all members of the zebra population in the area were at some time exposed to the virus. That is an indication that despite the dry and arid conditions, there is still a strong or at least seasonally consistent circulation of AHSV in the area to maintain antibodies in the whole population. There must be a viable reservoir source in the area.

Even though a greater number of zebra were found in the south-west of the study area, AHS was not very prevalent there. Locations which had the highest potential viremic zebra, with horse numbers comparable with those in lower potential viremic zebra zone, reported no cases of AHS. This was surprising, as the more cycling hosts there are, the greater the total viremia count is expected to be in an area. It must also be noted that these areas had a lower than average mean annual rainfall and may support fewer vectors than in areas that had a higher rainfall where there were fewer available viremic zebra.

Interestingly, during higher rainfall periods, the viremic source animals also appear to move further south-west, away from the locations with highest number of cases of AHS, although, at almost all locations, there were always at least a few zebras present (Table 3.6 and Fig. 3.9). Perhaps the number of available viremic zebra is not such a significant factor, rather that there were at least a few present, coupled with other necessary conditions.

From the data, it was confirmed that a zebra species (*E. z. hartmannae*) were numerous in the south-western Khomas Region, and that individuals were regularly infected with the AHSV as indicated by the presence of antibodies in specimens throughout the year. This is in accordance with findings in the literature on *E. burchelli* (Barnard, 1993). Of special interest was the presence of viral RNA in zebra blood during the dry season when midge numbers are expected to be few or non-existent (see section 3.3). It is concluded that these animals were certainly hosts for the AHSV in the area and since the *E. z. hartmannae* do not die from the disease, they will play some role in the perpetuation of the virus by serving, at least to some extent, as a cycling host or by forming part of the reservoir pool.

3.3. THE OCCURRENCE OF *CULICOIDES* SPECIES IN THE SOUTH-WESTERN KHOMAS REGION, NAMIBIA WITH SPECIAL REFERENCE TO POTENTIAL AS VECTORS OF AHSV

3.3.1. Introduction

To confirm AHS as endemic to the south-western Khomas Region, the area must support enough *Culicoides* species that serve as vectors to the disease. The virus must also be sustained in a local reservoir pool (Higgs & Beaty, 2005), which may include *Culicoides* midges. Adult *Culicoides* midges may survive the winter or maintain the virus by an interaction with a vertebrate reservoir (Barnard, 1993; Borkent, 2004) (see section 3.2).

There are 112 confirmed *Culicoides* species (estimated up to 120 species*),* divided into 38 subgenera in southern Africa of which *C. imicola* Kieffer is the only confirmed vector for AHSV (Meiswinkel *et al.,* 2004a). However, field-collected *Culicoides bolitinos* was found to be infected with AHSV and responsible for outbreaks in the cooler Free State, South Africa (Meiswinkel & Paweska, 2003), although it is yet to be proven that *C. bolitinos* can transmit the virus to susceptible hosts during blood feeding.

Due to their relative abundance near farm animals, host preference and wide distribution, a few more *Culicoides* species, such as *Culicoides subschultzei*, *Culicoides schultzei* and *Culicoides magnus* (Nevill *et al*., 1992) are considered as potential vectors of AHSV. From laboratory tests, a further eight *Culicoides* species were considered likely to be susceptible to infection by AHSV, namely: *Culicoides zuluensis, Culicoides brucei, Culicoides enderleini, Culicoides pycnostictus, Culicoides engubandei, Culicoides dutoiti, Culicoides bedfordi, Culicoides expectator* (Paweska *et al.,* 2003; Venter & Paweska, 2007; Venter *et al.,* 2009c; Venter *et al.,* 2010*).*

AHS is considered endemic to Namibia. However, although Enderlein (1908 cited in Meiswinkel *et al*., 2004b) described the first sub-Saharan *Culicoides* species in Namibia in 1908, detailed evaluations of *Culicoides* communities across the whole of Namibia are not well documented.

In particular, the occurrence of *Culicoides* midges in the south-western Khomas Region, is unknown, but was expected to be limited or absent, as the area is particularly dry (see section 1.1) – conditions thought unfavourable for *Culicoides* midges (Conte *et al.,* 2007).

An article based on this chapter has been accepted for publication in the *Veterinaria Italiana* journal. This work has also been presented at the XVII Congress of the Royal Entomological Society of Southern Africa.

3.3.1.1. Factors which influence Culicoides midge occurrence

Whether or not *Culicoides* midge communities can become established in an area, is dependent on the available moisture, since *Culicoides* juvenile stages are dependent on moisture in order to complete their development (Nevill, 1967). Even the eggs are not resistant to desiccation (Borkent, 2004) and must remain marginally moist. Reflecting this limitation, Conte *et al.,* (2007) found that *Culicoides spp.* are less abundant, on average, as aridity increases, which is why *Culicoides* midge occurrence in the south-western Khomas Region is expected to be limited in general.

Although they normally disperse only a few kilometres from their breeding sites, *Culicoides* midges are expected to be good dispersers since they use very temporary habitats and must be capable of finding new breeding sites away from the original site of emergence (Borkent, 2004). It is believed that they disperse over long distances on wind currents (Sellers *et al.,* 1977; Braverman & Chechik, 1996). Thus it is possible that midges are able to colonise the south-western Khomas Region from a wetter inland 'source' area when conditions become favourable, despite not being able to survive there during dry winters or particularly dry summers.

Relative humidity and temperature also affect adult survivorship (Baldet *et al.,* 2008). Generally, adult midge activity increases as temperature increases (provided humidity is sufficiently high). *Culicoides* species become most abundant in late summer (in the summer rainfall areas of southern Africa) (Meiswinkel *et al.,* 2004a), when warm and moist conditions were optimal. If *Culicoides* midges are present in the south-western Khomas Region, it was expected that the apex of their abundance will also occur during the summer months.

However, either temperature extremes act as a limiting factor on *Culicoides* adult survival. Adult *Culicoides* mortality may be high when, accompanied by drought, temperatures soar too high as was the case in the dry Saudi Arabian summer (Kheir, 2010). This may be the case in the south-western Khomas Region. On the other extreme, in South Africa, numbers of *C. imicola* drop dramatically after the first frost (Venter *et al.,* 1997; Meiswinkel *et al.,* 2004a; Baldet *et al.,* 2008). In Saudi Arabia, a winter-rainfall arid area, *Culicoides* activity ceased entirely during the winter season (Kheir, 2010).

However, Namibian winters are comparatively mild. Although at night-time, temperatures can fall below zero, the duration is generally short. During the daytime temperatures frequently rise above 20°C (Mendelsohn *et al.,* 2002). Where moderate to cool temperatures reduce activity, it can extend *Culicoides* adult life span (Meiswinkel *et al*., 2004a; Vorsprach *et al*., 2009). In South Africa where winters are mild and frost-free, adults may survive and even breed (Venter *et al.,* 1997). It is therefore not unlikely that smaller *Culicoides* adult populations may be sustained in winter provided they do not succumb to desiccation. In such a case, the vector-free periods in the south-western Khomas Region may not be long enough to successfully break AHSV transmission to the local susceptible vertebrate population (Mellor, 1994). According to the International Animal Health Organisation (OIE) a 'vector-free period' can be declared when less than five parous *C. imicola* and/or *C. bolitinos* are caught per day (Baldet *et al.,* 2008), Annex V of Regulation (EC) No 1266/2007. However, since zebra can remain viremic for up to 40 days (Barnard *et al.,* 1994), *Culicoides* vectors must be absent for at least that period of time.

If adult *Culicoides* midges are able to survive the winter, so too can the AHSV by inhabiting these infected adult midges. It may even be possible that infected older generation *Culicoides* adults survive the winter and act as reservoirs themselves. This has implications for possible outbreaks of AHS during favourable conditions in Namibia. It will also provide clues as to the epidemiology and over wintering strategy of AHSV and related arboviruses.

The proof of the presence and the abundance of *Culicoides* midges in the south-western Khomas Region, and during the winter months in particular, is therefore an important research objective, especially since AHS has been reported in this arid summer rainfall area.

As to local variations in *Culicoides* distributions, the conditions which favour the occurrence of breeding sites are affected by different local environmental conditions or microclimates. As discussed in section 3.1, heading 3.1.4, the south-western Khomas Region has variable environmental conditions, which are expected to affect *Culicoides* midge abundance and the species composition of communities at different sites.

Breeding substrates used are diverse and potentially include any moist substrate containing organic material in which larvae can tunnel and feed, such as, soil, rock crevices, dung, tree-hollows, fruits and rotting vegetation (Nevill, 1967; Meiswinkel *et al.,* 2004a). Some *Culicoides* species are specialist breeders and only utilise one type of substrate (Nevill, 1967). However, there may be species that are more adaptable to whatever substrate is available. Since the afore-mentioned moist substrates are expected to be sparsely distributed in the south-western Khomas Region (and to varying densities in different rainfall zones); it is expected to be reflected by the *Culicoides* species composition.

Vegetation growth is also expected to affect *Culicoides* midge species composition and occurrence. Vegetation density can affect the available sunlight. Some species favour breeding in patches which receive more sunlight (*C. imicola*), where others avoid it (Nevill, 1967; Braverman & Mumcuoglu, 2009). Both male and female *Culicoides* adults feed on nectar (Meiswinkel *et al*., 2004a; Borkent, 2004), as well as rest on vegetation. Therefore vegetation is expected to affect *Culicoides* midge distributions to some degree.

Local environmental conditions which favour *Culicoides* midges are most common in low-lying areas, such as coastal regions, marshes, riverine environments and valleys (Meiswinkel *et al.,* 2004a). It is believed that the characteristic relief, altitude, vegetation cover and average slope of such areas are associated with the environmental conditions which favour breeding conditions and adult survivorship. As described under section 2.1, riparian vegetation, pools, soil characteristics and average slope along ephemeral rivers in the south-western Khomas Region may provide such favourable local conditions.

Whilst *Culicoides* males feed on plant sap and nectar, most females are also obligate blood-feeders, as required for the development of eggs (Mellor *et al.,* 2000). Distributions of female *Culicoides* midges should therefore also be dependent on the distribution of their hosts (Borkent, 2004). Some species, such as *C. imicola*, have a wide vertebrate host range; others, like *Culicoides ravus* feed primarily on birds (Meiswinkel, 1996 (unpublished)). It is expected that the distribution of hosts will also affect the distribution of *Culicoides* species and the species composition of a particular location. In the proximity of livestock, *C. imicola* can become superabundant under favourable conditions, in which case millions may be caught with a light trap in a single night (Meiswinkel *et al.,* 2004a). As the distribution of vertebrates are variable in the south-western Khomas Region, *Culicoides* midge occurrence is expected to be affected accordingly.

The objective of this part of the study was to determine the presence, species composition, richness and diversity of *Culicoide*s adults during the colder drier months in the south-western Khomas Region. The collection of midges over the dry winter months will represent the baseline value of *Culicoides* occurrence and distribution. This will serve as an indication of whether or not AHS can be maintained in the area and whether or not *Culicoides* midge numbers are likely to increase from a local source to cause outbreaks of AHS during favourable periods.

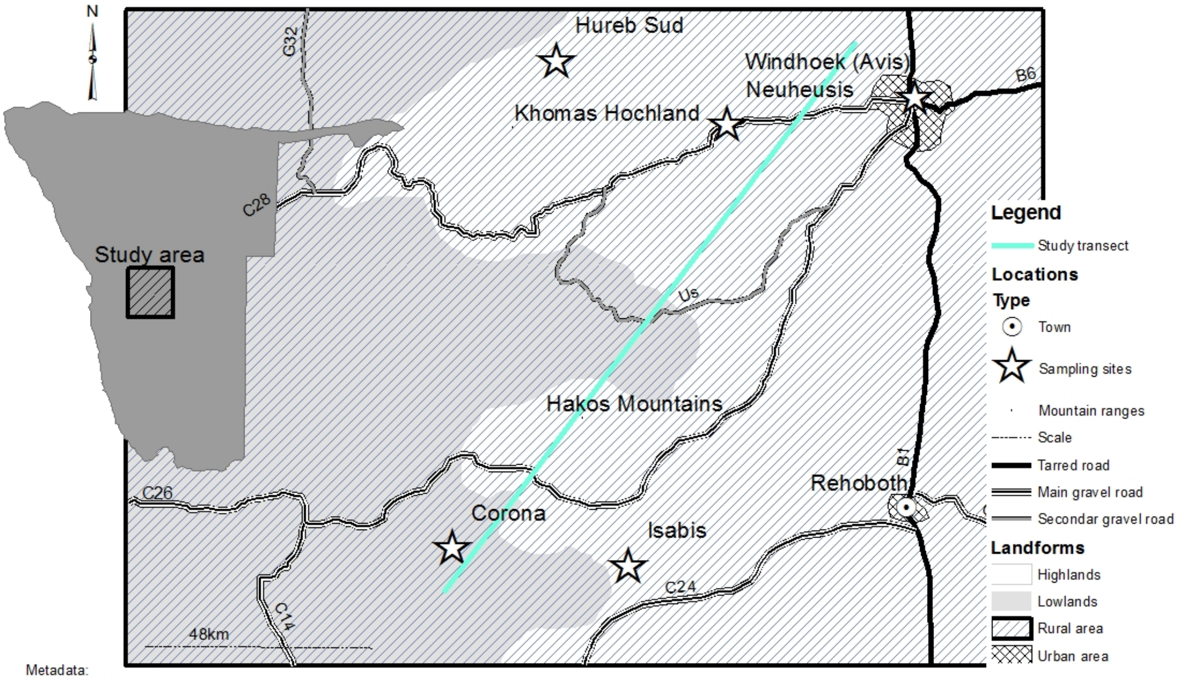
3.3.2. Materials and Methods

3.3.2.1. Study area

The area selected for sampling is shown in Fig. 3.11 as the area that was also surveyed by use of a questionnaire (see paragraph 3.1.2.2). It is delineated by the co-ordinates: 22° 24.063´ S, 17° 01.791´ E; 23° 32.617´ S, 15° 53.870´ E, which represents a transect through several rainfall zones (Fig. 3.1). The area is undulating with rolling hills and fold mountains (Mendelsohn *et al.,* 2002). See also section 2.1.

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 the date of their occurrence.

Refer also to section 3.1, which deals extensively with the study area.



Metadata:
Created by Elbè Becker
Google Earth (TM) satelite image
Garmin GPS waypoints
C:\Arc Catalog\Study area

Figure 3.11. The location of farms where *Culicoides* midge collections were made in the south-western Khomas Region, from 6 July 2009 to 21 September 2009.

3.3.2.2. Culicoides midge collections sites

Collections were made at five sites (Fig. 3.11). With the exception of the Avis horse stables in Windhoek, all the collection sites were located in rural settings. Attempts were made to install the trap at sites which resembled one another with regard to environmental variables (other than annual rainfall), such as trap height (see also Venter *et al*., 2009a) the proximity to stables, kraals, and host animals - particularly *E. z. hartmannae*.

The locations where the different traps were installed and operated, are shown in Fig. 3.11, with 420 mm/a as the highest rainfall point at Neu Heusis and the lowest (180 mm/a) at Corona. The intermediate rainfall points on the gradient line are Hureb Süd (250 mm/a) and Isabis at 220 mm/a (see also Table 3.1). As determined by a telephonic survey, no rainfall was reported by farm owners during the winter months 6 July to 21 September 2009.

The sites were not chosen to fit the rainfall gradient line exactly, but rather those that presented a reasonable rainfall interval. A dependable electricity source was also requisite.

Neu Heusis.

Farm Neu Heusis is situated about 40km west of Windhoek. Neu Heusis homestead site (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, remaining stabled overnight. About ten to fifteen horses roamed free in surrounding paddocks. The trap was affixed to the stable within five meters of the stable door and about 10 m from the paddocks. 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 was 420 mm/a (landowner’s communication).

Avis stables

The Avis horse stables site (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 were able to stand outside at night in small encampments in front of their stalls The trap was affixed to an out-building within three meters 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 rainfall was estimated at 360 mm/a.

Windhoek was atypical of the rest of the locations due to the fact that there were considerably more horses stabled in a small area for most of the hours of the day. It is also surrounded by a matrix of anthropogenic areas, whereas the other locations were surrounded by undeveloped, natural areas. It was also atypical in that *E. z. hartmannae* were few and did not tend to approach human inhabited areas.

Hureb Süd

Farm Hureb Süd is 80 km from Windhoek. The landscape, in which Hureb Süd is situated, is very undulating. Hureb Süd homestead site (22° 29.394´S; 16° 22.172´E) is at an altitude of 1 216 m above sea-level and situated within the bluff line of an ephemeral river abounded by hills and spurs, which had not flowed for more than a year - the entire time period of this study.

The trap was installed in the homestead garden. Several vegetation-free paddocks, some 20 m from the trap, occasionally held prize horses. Fifteen meters from the trap, there were two stables where horses were seldom kept – except mares with their foals and/or sick horses. Horses were bred on the farm and therefore numbered more than 50 animals which roam free on 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 in the area. 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 was 250 mm/a (landowner's communication).

Isabis

The farm Isabis is situated 113 km south-west of Windhoek. Isabis homestead site (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 six meters) 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. There were no *E. z. hartmannae*.

No water spillage was observed in the surrounding area during the study period. The garden, some 50m 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 was 220 mm/a (landowner's communication).

Corona

Farm Corona is situated 130 km south-west of Windhoek. 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. The average slope is gentle and the landscape slightly undulating. The homestead is backed by steep mountains to the north-east.

There were no stables, but the trap was affixed to the side of a building which faces a large wildlife-enclosure with a water trough (some 20 m from the trap), which horses frequent. Horses are the only domesticated animals kept on the farm. About 20 to 25 horses roam free on the farm. Water seepage from an irrigation pipe into garden-refuse was observed some five meters from the trap. The nearby garden (15 m from the trap) was lush and watered regularly. The estimated annual rainfall at Corona was 120 mm/a (landowner's communication).

3.3.2.3. Equipment and Culicoides midge collection procedure

1. Suction UV-light midge-trap
2. Collection beaker
3. Sock
4. Sieve
5. Distilled water squirt bottle
6. Specimen bottles with ethanol
7. Calendar: the seasonal occurrence of *Culicoides spp*. along a moisture gradient
8. Ethylene glycol preservative
9. Electricity power supply
10. Time switch
11. Minimum-maximum thermometer

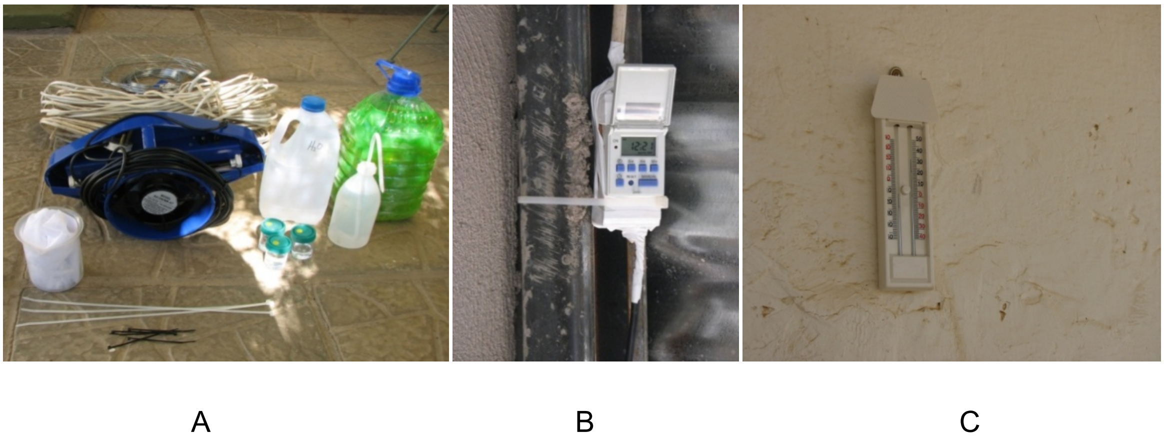


Fig. 3.12. *Culicoides* species collection equipment. A suction UV-light trap and components, B Timer switch, C Minimum-maximum thermometer.

(a) Installation of UV-light traps

Collections were made during the winter season from 6 July to 21 September 2009 using the Onderstepoort 220V suction UV-light trap as described by Venter *et al.,* (2009b). Five suction UV-light traps were installed along the gradient (Fig. 3.11) where there is an electricity supply. The traps were all set up to be exposed at 180° to the outside environment. Traps were installed at the same height relative to the ground as consistently as possible; however, this was ultimately dependent on the sites that were available and the possibility of tampering by animals.

Most species of southern African midges are diurnal (Meiswinkel *et al*., 2004a), preferring to fly under warm and calm conditions; the traps were therefore only operated between sunset and sunrise. The operation duration was regulated by a Toptronic® programmable time switch (model TDDT7). The timer switch synchronised the traps, so that they all switched on at 17h00 pm and switched off at 06h00 am.

The traps were emptied once per week by the farmers on whose property the traps were installed (see 3.3.2.2). Collections were made in 30% ethylene glycol solution to preserve the samples to the week's end. Samples were transferred to 70% ethanol solution, wherein the specimens were preserved (Goffredo & Meiswinkel, 2004). At the ARC-Onderstepoort Veterinary Institute, the number of *Culicoides* midges in each sample was determined and identified to species level (see 3.3.2.3 (b)).

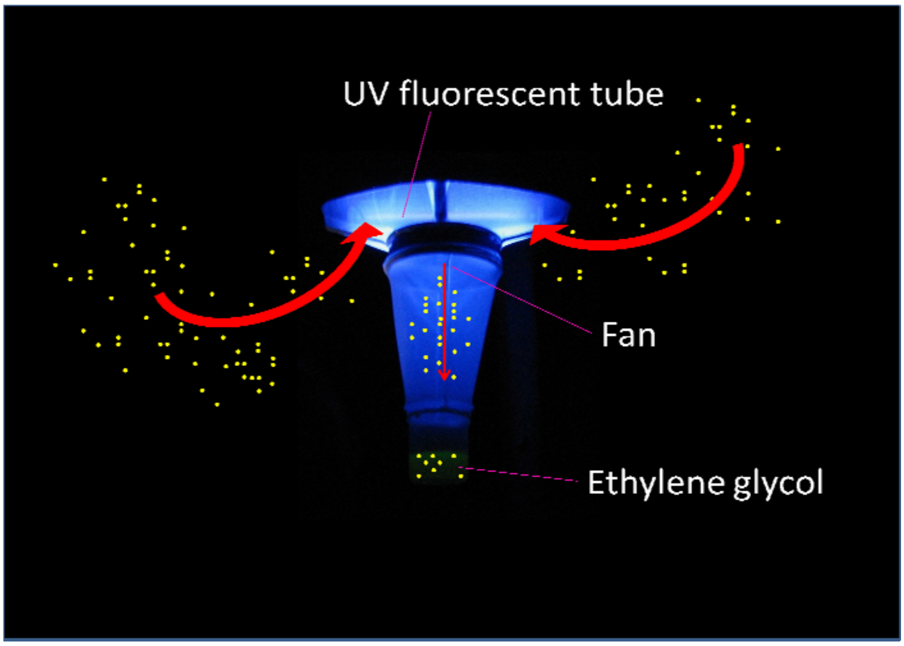


Figure 3.13. Diagrammatic illustration of how the trap operates.

The suction UV-light trap (Fig. 3.13) operates by a suction mechanism caused by a fan (Venter *et al.,* 2009b). At night, the midges are attracted to the UV-fluorescent lamp, fly through the gauze with 4mm aperture (Goffredo & Meiswinkel, 2004) and are then blown into the 500ml plastic beaker below, which contains the 30% ethylene glycol preservative. The gauze is positioned over the UV-fluorescent tubing, to filter out large insects which could contaminate the catch. The specimens are stored in 70% ethanol (Goffredo & Meiswinkel, 2004) and transported in screw-top containers. Each week's catch was identified by the accompanying label, documenting place and date of capture.

(b) Identification of *Culicoides* midge species

At the ARC - Onderstepoort Veterinary Institute 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).

Species were identified by use of a stereo microscope. In addition to wing pattern, other morphological characteristics were used, such as the shape of spermathecae, the eye-space, the absence/presence of a sensory pit on the third palpal segment, and the number of the coeloconicae per antennomere (Meiswinkel *et al.,* 2004a & b).

3.3.2.4. Data analysis

Culicoides midges collected weekly per location over the winter were averaged for comparison, and standard deviation calculated as a measure of inter-weekly variation in numbers. The results are summarised in Table 3.8.

The composition of species and percentage representation of each *Culicoides* species per site were calculated as a percentage of the sum per site, as well as the percentage of the sum of all sites. The results are summarised in Table 3.9.

Microsoft Excel ® software was used to tabulate the data and calculate percentages.

The number of *Cullicoides* species and abundance per species at each site was used to determine species richness (Margalef index) (Margalef, 1958), which takes sample size into account. Species diversity was calculated with the Shannon index (Shannon & Weaver, 1949), which describes the species richness as well as 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). The results are summarised in Table 3.10.

The equations of the diversity indices are as follows:

Margalef index (d):

$$d = (S - 1)/\ln N$$

Where:

S = the total number of species

N = number of individuals

Shannon index (H'):

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

Where:

S = the total number of species

p_i = frequency of the ith species

The occurrence patterns of *Culicoides* midges were compared with the rainfall data collected in Section 2, as well as the occurrence of *E. z. hartmannae* demonstrated in Section 3.

3.3.3. Results

From 6 July to 21 September 2009 34 light trap collections were made at the five trap sites and 9091 *Culicoides* individuals belonging to 25 species were collected. The mean number of *Culicoides* species collected weekly at each site is shown in Table 3.8. The relative species composition and abundance collected at each site for the sampling period are shown in Table 3.9. The total number of species, individuals, species richness and Shannon diversity indices for each site are shown in Table 3.10.

Table 3.8. The mean weekly *Culicoides* species collections made in suction UV-light traps at five sites in the Khomas Region, Namibia, from 6 July to 21 September 2009

Location	Avis	Neu Heusis	Hureb Süd	Isabis	Corona
Culicoides species:					
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 per week	235	66	410	95	597

Table 3.9. The relative *Culicoides* species composition from collections made in suction UV-light traps at five sampled sites in the Khomas Region, Namibia, from 6 July to 21 September 2009.

Site	Avis		Neu Heusis		Hureb Süd		Isabis		Corona	
No. of collections made	6		10		6		4		8	
Species	% 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
Culicoides cornutus					0.4	*				
C. expectator	0.1	*			5	1.6			*	*
C. herero	0.2	*	0.3	*	6.1	1.9	1.3	0.1	9	4.1
C. imicola	93.9	16.6	19	0.9	20.2	6.2	27.4	2	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	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			*	*
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									*	*
% 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 3.10. The total number of *Culicoides* species, individuals, richness and diversity indices from suction UV-light collections made at five sampled sites in the Khomas Region, Namibia, from 6 July to 21 September 2009.

Site	**Neu Heusis**	**Avis**	**Hureb Süd**	**Isabis**	**Corona**
Total individuals (N)	657	1 412	2 462	379	4 181
Total species (S)	14	11	15	10	20
Species richness (d)	2.004	1.379	1.793	1.516	2.279
Shannon's Diversity (H')	1.543	0.3236	1.605	1.475	1.521

Neu Heusis

The lowest number of midges was collected at Neu Heusis (Table 3.8) and it accounted for only 5.2% of the total number of midges collected in this survey (Table 3.9). A total of 657 *Culicoides* midges were collected over a period of ten weeks (Table 3.10); that is about 66 *Culicoides* individuals per week (Table 3.8). The dominant species was *Culicoides ravus* which comprised 36.2% of the collection. *Culicoides subschultzei* had the second largest representation at 23.0% of the site catch and 1.1% of the total number of *Culicoides* collected (Table 3.9). Despite the low numbers collected 14 species were represented in the collections, resulting in a diversity index of H' = 1.543 and a richness index of d = 2.004 (Table 3.10). This was the only sight 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 traps.

Avis

At Avis 1 412 *Culicoides* midges comprising 11 species were collected over six weeks (Table 3.10), at about 235 *Culicoides* midges per week (Table 3.8). Of the 11 species collected, *C. imicola* dominated at 93.9% of the collection and accounted for 16.6% of all *Culicoides* species collected during this survey (Table 3.9). 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 3.9). 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 3.10). There were no zero values during weeks of operation.

Hureb Süd

Hureb Süd was the second most productive site and 2 462 *Culicoides* were collected over a period of six weeks (Table 3.10); that is about 410 *Culicoides* midges per week (Table 3.8). Of the 15 species collected the dominant species was *C. ravus* representing 44.9% of the site collection and accounted for 13.9% of the total number of *Culicoides* midges collected in this survey (Table 3.9). It was seconded by *C. imicola* comprising 20.2% of the site collection and 6.2% of the total *Culicoides* midge collection (Table 3.9). The diversity index for Hureb Süd was H' = 1.605 and the richness index d = 1.793 (Table 3.10).

Isabis

At Isabis, a total of 379 *Culicoides* were collected over a period of four weeks (Table 3.10), therefore about 95 *Culicoides* individuals per week (Table 3.8). Ten species were represented at Isabis (Table 3.10). The dominant species was *C. ravus* representing 46.7% of the site collection and 3.3% of the total number of *Culicoides* midge collected. It was seconded by *C. imicola* at 27.4% and 2.0% of the total *Culicoides* collection (Table 3.9). Isabis had a diversity index of H' = 1.475 and a species richness index of d = 1.516 (Table 3.10).

Corona

The biggest collections were made at Corona and 4 181 *Culicoides* midges were collected over a period of seven weeks (Table 3.10); that is an average of 597 *Culicoides* midges per week (Table 3.8). Twenty species were represented at Corona (Table 3.10), 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 is seconded by *C. imicola* at 12.3% of the site collection and 5.5% of the total *Culicoides* midge collection (Table 3.9). The diversity index was H' = 1.521 and the species richness index was d = 2.279 – the highest of all the sampled sites (Table 3.10).

3.3.4. Discussion

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. To put these results into perspective, it also must be kept in mind that suction UV-light traps collections represent less than 0.0001% of the midge population (Venter *et al.,* 2009b) and therefore *Culicoides* midge numbers may be far more than the impression created from suction UV-light trap collections.

At all the sites, except Avis, the dominant species was *C. ravus* (Table 3.9), followed either by *C. imicola* or *C. subschultzei*. *Culicoides 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%). *Culicoides 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*, *Culicoides herero*, *Culicoides leucostictus*, *Culicoides pycnostictus*, *Culicoides subschultzei* and *Culicoides 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), *Culicoides kanagai*, *Culicoides #89*, *C. magnus*, *Culicoides olyslageri*, *Culicoides remerki* and *Culicoides similis* showed a more limited or patchy distribution (Table 3.9). Although *Culicoides expectator*, *C. herero*, *Culicoides tuttifrutti*, *Culicoides macintoshi*, *Culicoides 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 have a low percentage representation were: *Culicoides cornutus*, *Culicoides pretoriensis*, *Culicoides schultzei*, *Culicoides bedfordi*, *Culicoides #94*, *Culicoides brucei*, *Culicoides nivosus* and a *Culicoides* species belonging to the Accraensis group (Table 3.9).

At the Avis stables, diversity was low (Table 3.10) and *C. imicola* was strongly dominant with no clear secondary dominant species (Table 3.9). Avis' environment was also different from the other sampled sites, as it was located in an urban area. The site's low diversity (H = 0.3236) coupled with the strong *C. imicola* dominance (the species richness index was only d = 1.379) may indicate disturbance in the natural ecology of *Culicoides* species due to anthropogenic urban environment. Further research is underway to address this question.

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. Avis had the highest host densities, yet also the lowest *Culicoides* species diversity – supporting the previous findings by Meiswinkel *et al*. (2004a) 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. There are also 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 = 1.793, 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 gentler topography, Neu Heusis and Isabis, showed lower total *Culicoides* midge collections than the lower altitude locations, Hureb Süd and Corona (Table 3.10). It is possible that the altitude and topography affects availability of breeding sites and the distribution of wild animals which may be potential hosts. *E. z. hartmannae*, for instance, prefers the more rugged terrains (Cillié, 2004) which characterises Hureb Süd and Corona.

In terms of total *Culicoides* midge numbers, there was no discernable link to the rainfall and total *Culicoides* midges collected (Table 3.8). Avis was considered an outlier in terms of its representation of a rainfall zone, since it is located in an urban environment. Neu Heusis which is situated in the highest rainfall zone had the lowest *Culicoides* midge count. Corona which falls in the lowest rainfall zone, showed the highest *Culicoides* midge count; and Hureb Süd and Isabis again show the reverse. Species heterogeneity was slightly greater in the higher rainfall zones during winter, although not significantly so.

Culicoides midge activity did not cease over the dry season in the south-western Khomas Region and its species richness was high compared with higher rainfall regions, such as the Stellenbosch area (Venter *et al.,* 2006) 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 winter months in the current study.

At least five of the species collected in this study, *C. imicola*, *C. pycnostictus*, *C. subschultzei*, *C. schultzei* and *C. magnus* are considered high potential orbivirus vectors, based on their host preference and virus isolation from field collected samples from collections made across South Africa (Nevill *et al*., 1992).

Although water usage and/or spillage at all locations were by no means profuse, the available soil moisture was expected to be more than it would have been if the sites had 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 or not significant differences in *Culicoides* midge numbers exist between homestead and veld sites.

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 and surveys to confirm this are underway. The high species diversity found and the presence of a number of species of which little is known about their biology and potential as vector for arboviruses, will add to the complexity of the epidemiology of AHSV in Namibia and indicates an important field of further research.