2. MATERIALS AND METHODS

2.1. Study area

The south-western Khomas Region is located in the western escarpment of Namibia, in the districts of Solitaire, Rehoboth and Windhoek (Fig. 2.2). This area was chosen for this study as AHS was reported to be present there, and for its apparent contrasting environmental conditions within a relatively small area (50 000 km^2). A study transect was selected (line indicated in blue in Fig. 2.2 and Fig. 3.1) along which a gradient of rainfall was expected and may therefore represent a variety of habitats which could be compared with one another. The transect cuts across a rugged, rocky terrain (Fig. 2.1), with a drop in elevation of approximately 2 100 m to 1 000 m in the east-west direction. It also cut across two ephemeral rivers. The area is semi-arid to arid with patchy and unpredictable rainfall (Mendelsohn *et al.,* 2002).

The distance covered (represented by the blue line) is approximately 172 km and the points on the suggested study transect are delineated by the co-ordinates: 22° 24.063´ S, 17° 01.791´ E; 23° 32.617´ S, 15° 53.870´ E.

Figure 2.1. Hartmann's mountain zebra (*E. z. hartmannae*) in the rugged terrain of the south-western Khomas Region

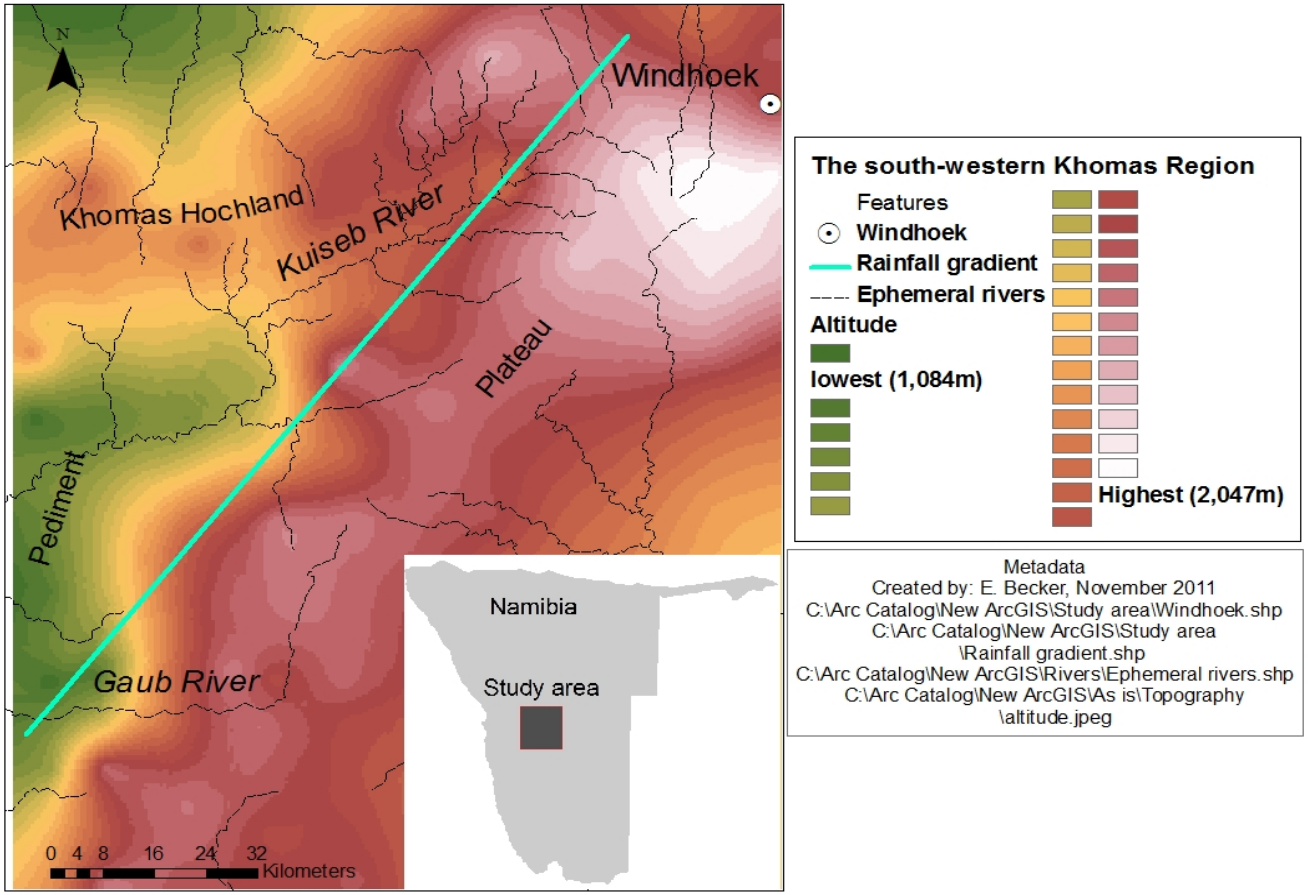

Figure 2.2. Enlarged map of the study area.

The study area was located in a mostly rural setting aside from the city of Windhoek, which has a population of 233 529 people (according to the most recent published census (Swartz, 2001)). The rural south-western Khomas Region is sparsely populated (Mendelsohn *et al.,* 2002), with the primary economic activities based on cattle farming, horse breeding and private reserves/guest farms (personal observation). Wild animals such as Hartmann's mountain zebra (*E. z. hartmannae*), kudu (*Tragelaphus strepsiceros*) and oryx (*Oryx gazelle*) are indigenous to the area. No large scale crop plantations were present in the area and therefore there were no large-scale irrigation practices.

The south-western Khomas Region can be divided approximately into three zones: the plateau, escarpment and pediment. Joubert (1973) described the escarpment of which the study area forms a part, as a transitional zone between the inland plateau to the pre-Namib pediment in the east. The plateau and Khomas Hochland is characterised by highland savannah (Giess, 1971 as quoted by Joubert, 1973). The escarpment is drained exoreically (rivers that drain away to the sea) by the Kuiseb, Oanob and Swakop River basins, although rainfall is often so low that the ephemeral flows of the river rarely actually reach the sea. However the river beds are dotted with permanent to semi-permanent rock pools. Several other smaller ephemeral rivers end blindly inland (endorheic rivers) in dunes or inland pans (Joubert, 1973). The escarpment vegetation is different from the plateau and is dominated by shrubs, bushes, *Euphorbia virsosa* (gifnoors), and trees more accustomed to dry conditions, such as *Aloe dichotoma* (quiver tree). The escarpment vegetation also consists of several grass species not present on the plateau, and the tufts are generally smaller. The pre-Namib pediment vegetation has an even greater dominance of shrubs, with trees sparsely distributed. The grass variation here however is very low. Riparian vegetation (Joubert, 1973) is distinctly different from the surrounding areas, and support tall trees, which often form a closed canopy.

2.2. Data gathering and analysis

The study was conducted from 6 July 2009 to 6 October 2010. Follow-up surveys made during the end of 2011 to verify trends in rainfall and occurrence of AHS in horses.

(i) To fill some information gaps regarding the occurrence of AHS, geomorphology and the climate of the south-western Khomas Region, a questionnaire was used to gather expert opinion from farmers in the area. The questionnaire was conducted telephonically. Rainfall data from Isabis farm in the area dating back 38 years was obtained from the farmer to illustrate the potential variability of rainfall in the area and

how the occurrence of hosts and vectors of AHS, thought to be dependent on rainfall, may vary from what is observed over this study period (see section 3.1).

Regression techniques were used to determine whether or not the number of cases of AHS can be standardised to incidence proportion (see 3.1.2.4(a) and 3.1.2.4(b)). The incidence proportion relationship with the annual rainfall for the years 2009/2010 and 2010/2011 in the south-western Khomas Region was analysed by use of regression techniques. These calculations were made by use of STATISTICA software of StatSoft Inc. Various environmental conditions obtained from the questionnaire were represented spatially (see 3.1.2.4(c)) in conjunction with the occurrence of AHS incidence proportion and *E. z. hartmannae*. These maps were drawn by use of the Google EarthTM application and ESRI® ArcGIS® software.

(ii) The same farms that were sampled for (i) above, were surveyed for the occurrence of *E. z. hartmannae* in the area and their migration habits to discern whether or not this potential cycling host is present in sufficient numbers to 'source' a virus challenge in the areas over which they are distributed (see 3.2.2.2). The same questionnaire was used as in (i) above.

(iii) To prove that the *E. z. hartmannae* are infected with the AHSV; and can potentially act as reservoir in the area, zebras shot by trophy-hunters and for meat-rations were sampled for blood and tissue (see 3.2.2.3). These samples were analysed for anti-AHSV antibodies by ELISA's, viral RNA by RT-PCR and viable viruses by viral isolation from cell culture inoculations (see 3.2.2.4).

(iv) To determine whether or not AHS overwinters in the south-western Khomas Region, the occurrence of *Culicoides* species were evaluated at five sites by use of suction UV-light traps along the selected study transect, each situated in different rainfall zones, ranging from 420 mm/a in the north east to 120 mm/a in the south west of the study area (see section 3.3).

(v) Microsoft Excel ® and STATISTICA software (see 3.1.2.4, 3.2.2.5 and 3.3.2.4) were used to conduct basic statistical analyses and PRIMER 5 software for Windows, version 5.2.9 (Copyright 2002 PRIMER-E Ltd) (see 3.3.2.4) was used to determine biodiversity and dominance of *Culicoides* midge communities between sites.