Aus dem Institut für Epidemiologie des Friedrich-Loeffler-Instituts

eingereicht über das
Institut für Mikrobiologie und Tierseuchen
des Fachbereichs Veterinärmedizin
der Freien Universität Berlin

RABIES IN GREATER KUDU ANTELOPE IN NAMIBIA

History, Epidemiological Studies and Vaccinology Experiments

Inaugural-Dissertation
zur Erlangung des Grades eines
Doktors der Veterinärmedizin
an der
Freien Universität Berlin

vorgelegt von
Rainer Hubertus Hassel
Tierarzt aus Windhoek, Namibia

Berlin 2019
Journal-Nr.: 4110

mbvberlin | mensch und buch verlag

Aus dem Institut für Epidemiologie des Friedrich-Loeffler-Instituts

eingereicht über das
Institut für Mikrobiologie und Tierseuchen
des Fachbereichs Veterinärmedizin
der Freien Universität Berlin

RABIES IN GREATER KUDU ANTELOPE IN NAMIBIA

History, Epidemiological Studies and Vaccinology Experiments

Inaugural-Dissertation
zur Erlangung des Grades eines
Doktors der Veterinärmedizin
an der
Freien Universität Berlin

vorgelegt von
Rainer Hubertus Hassel
Tierarzt aus Windhoek, Namibia

Berlin 2019
Journal-Nr.: 4110

Gedruckt mit Genehmigung des Fachbereichs Veterinärmedizin

der Freien Universität Berlin

Dekan:	Univ.-Prof. Dr. Jürgen Zentek
Erster Gutachter:	Prof. Dr. Franz J. Conraths
Zweiter Gutachter:	Univ.-Prof. Dr. Heribert Hofer
Dritter Gutachter:	PD.Dr. Nicolai Denzin

Deskriptoren (nach CAB-Thesaurus):
Antelope, Rabies, Zoonoses, Vaccination, Epidemiology, Namibia

Tag der Promotion: 01.07.2019

Bibliografische Information der *Deutschen Nationalbibliothek*
Die Deutsche Nationalbibliothek verzeichnet diese Publikation in der Deutschen Nationalbibliografie; detaillierte bibliografische Daten sind im Internet über <https://dnb.de> abrufbar.

ISBN: 978-3-96729-008-0
Zugl.: Berlin, Freie Univ., Diss., 2019
Dissertation, Freie Universität Berlin
D188

 Choriner Str. 85 - 10119 Berlin
verlag@menschundbuch.de – www.menschundbuch.de

This work is dedicated to

My friend, the late Theuns van Wyk, game capture veterinarian in the Department of Nature Conservation and Tourism, who supported and assisted me with the experimental work related to rabies in kudu in the 1970' s and early 1980's and who passed away much, much to early, suffering from cancer;

My family who means everything to me and who has always supported me in all my endeavours as veterinarian, throughout my professional career in our beloved Namibia;

All the Namibian farmers who suffered and are still suffering horrendous losses as a result of rabies in our beautiful iconic kudu antelope,

With the promise, that I shall continue with my modest contribution towards finding a solution for this scourge.

Table of Contents

List of Standard Acronyms

AHT	**Animal Health Technician**
BVDV	**Bovine Virus Diarrhoea Virus**
CANAM	**Conservancies Association of Namibia**
CBPP	**Contagious Bovine Pleuropneumonia**
CVL	**Central Veterinary Laboratory (Namibia)**
CVO	**Chief Veterinary Officer (Namibia)**
DALY	**Disability Adjusted Life-Year**
DFA	**Direct Fluorescent Antibody (test)**
DRF	**Disease Report Form**
DRIT	**Direct Immunohistochemical Test**
DVS	**Directorate of Veterinary Services (Namibia)**
ELISA	**Enzyme Linked Immunosorbent Assay**
FAT	**Fluorescent Antibody Test**
FLI	**Friedrich-Loeffler-Institut (Germany)**
FMD	**Foot-and-Mouth Disease**
GARC	**Global Alliance for Rabies Control**
HEP	**High Egg Passage**
IUCN	**International Union for Conservation of Nature and Natural Resources**
LPO	**Livestock Producers Organization (Namibia)**
MAWF	**Ministry of Agriculture Water and Forestry (Namibia)**
MET	**Ministry of Environment and Tourism (Namibia)**
NAPHA	**Namibia Professional Hunting Association**
NAU	**Namibia Agricultural Union**
NCAs	**Northern Communal Areas**
OIE	**World Organization for Animal Health**
PARACON	**Pan-African Rabies Control Network**
RABV	**Rabies Virus**
RAPINA	**Rapid Neutralizing Antibody (test)**
RFFIT	**Rapid Fluorescent Focus Inhibition Test**
RT-PCR	**Real Time Polymerase Chain Reaction**
RVL	**Regional Veterinary Laboratory**
SADC	**Southern African Development Community**
SEARG	**Southern and East African Rabies Group**
SWA	**Southwest Africa**
VCF	**Veterinary Cordon Fence**
WHO	**World Health Organization**

1. Introduction and Background

1.1 Namibia, the Greater Kudu and Rabies

Namibia is a country rich in a wide range of wildlife resources attracting thousands of tourists annually from all over the world. However, since 1977 two epidemics of rabies occurred in one of the major, most popular and most iconic species of antelope, the Greater Kudu, *Tragelaphus strepsiceros*. During the first epidemic from 1977 to 1986 approximately 30 000 to 50 000 kudu or 20 – 40% of the total population died of rabies (Barnard & Hassel, 1981; Hassel; 1982; Hübschle, 1988; Schneider, 1994). Between 1977 and 2017, 1065 cases of rabies were laboratory confirmed in this antelope species and there is no indication that the intermittent outbreaks of the disease will subside any time soon (Hassel, 1980; Hassel, 2011).

Veterinary services in Namibia need to respond to their mandate to ensure the maintenance of animal health, including wildlife, as well as the prevention or control of zoonotic diseases. Tourism including trophy hunting of game is an important and substantial economic factor and source of foreign currency for Namibia. The domestic consumption of game meat or venison also serves as a common source of protein for the Namibian population.

For these reasons the Livestock Producers Organization of the Namibia Agricultural Union passed a resolution at its Annual General Members Meeting in 2010, calling for: "*find a solution to the kudu rabies problem with the aid of a suitable vaccine".* This in turn led to the author being contracted to draft a project proposal and subsequently to implement **"An Epidemiological Survey of Rabies in Kudu and the Development of an Oral Anti-Rabies Vaccine for Kudu in Namibia."** In short, it is referred to as the "Kudu Rabies Research Project" and contains objectives related to the epidemiology of rabies in kudu as well as vaccinology. This project forms the basis for this dissertation.

Rabies in kudu antelope is unique and does not occur outside Namibia in other area where kudu occur, nor does it affect other game species in Namibia or southern Africa to the same extent, although an increase in the number of cases in eland antelope, *Taurotragus oryx,* has been recorded as well. Reasons as to why the kudu, and also the eland, appear to be exceptionally susceptible to the disease still remain speculative at best. At one time, it was postulated that the kudu might be immune-compromised, possibly related to BVDV infections as indicated by a high prevalence of 71% of circulating anti Bovine Virus Diarrhoea virus (BVDV) antibodies, since BVDV is known to be immunosuppressive in other species (Depner et al., 1991; Scott et al., 2013). Current vaccine trials involving the intra-muscular administration of an inactivated rabies vaccine have, however, shown that the immune system of healthy kudu can mount a strong immune response, able to protect the animals against subsequent viral challenges.

1.2 Objectives of this Thesis

The objectives of this thesis are in line with the objectives and activities of the current kudu rabies research project: "**An Epidemiological Survey of Rabies in Kudu and the Development of an Oral Anti-Rabies Vaccine for Kudu in Namibia."** These objectives are:

1. To improve the knowledge regarding the epidemiology of rabies in kudu antelope in Namibia
2. To investigate the possible existence of active natural immunity to rabies in individual free-ranging animals
3. To prove the possibility of transmission of rabies between individual animals by direct contact via infectious saliva, thus proving the possibility of lateral spread of the disease in the kudu population
4. To develop a safe, effective and practical method of oral vaccination of kudu antelope against rabies.

In addition to the objectives and activities of the current project, the objectives and results of previous research activities conducted between 1980 and 1983 are also discussed, including epidemiological studies, vaccine experiments and experimental infection. As a result of this combination of the historic aspects of rabies in kudu with the current study, a complete picture of this unique phenomenon should emerge that will hopefully guide future endeavours aimed at controlling the disease.

It should also be pointed out here that the objectives of the current as well as the historic studies are perfectly aligned with the requirements for conducting research in terms of subsection 3.3.2(4) of the Rabies Control Strategy, as described in Chapter 2.5.4.4 of this dissertation.

2. Literature Review

2.1 Namibia as Study Area

Namibia, situated in the south-western corner of the African continent, is a large country of about 823 680 sqkm. It spans 1 320 km at its longest and 1 440 km at its widest points. The coastline of approximately 1 570 km separates the land from the southern Atlantic Ocean (Mendelsohn et al., 2009). Namibia shares its borders with Angola, Zambia, Zimbabwe, Botswana and South Africa. Namibia was given its current name upon independence from South Africa in 1990. Prior to independence, from 1884 until July 1915, it was known as *"German South-West Africa"* (Deutsch-Südwestafrika) during the German colonial occupation, from 1915 until 1977 as *"Southwest Africa"* (SWA) during the period of South African administration and from 1978 until 1990 as *"SWA/Namibia"* during the period of the Transitional Government. To avoid confusion only the term *"Namibia"* will be used to describe the territory for the whole period under review in this dissertation, including the colonial and pre-independence periods.
After independence in 1990, the names of districts in Namibia changed and the concept of different regions was introduced. The area of Namibia situated north of the Veterinary Cordon Fence (VCF) (Figure 1) is referred to as the *"Northern Communal Areas" (NCAs),* which include – from west to east – the regions *"Kunene", "Omusati", "Oshana", "Ohangwena", "Oshikoto", "Kavango West", "Kavango East", and "Zambesi"* (Figure 2). The so-called North-Central or "4-O- Regions" (Omusati, Oshana, Ohangwena and Oshikoto) constitute what was previously known as *"Ovamboland"* (Figure 2). Since August 2013 Namibia has 14 regions because Kavango was divided into Kavango West and Kavango East. At the same time, the Caprivi region was renamed Zambesi region. Part of the Kunene region in the west lies north of the VCF, while the rest is situated south of the VCF.

Today, Namibia supports in excess of two million head of plains game. This figure is roughly similar to cattle, sheep and goats (van Schalkwyk, 2016). Wildlife in Namibia attracts tourists and trophy hunters, is sold as live animals, harvested for commercial meat production and for own on-farm use (van Schalkwyk, 2016). Combined wildlife use contributes an estimated 3.5% to the Gross Domestic Product (GDP) compared to 3.2% contribution by agriculture, 2.4% by the fishing industry, 13% by mining and 13.3% by the manufacturing sector. Meat processing accounts for a further 2.3% of the GDP. These figures are based on calculations by the Namibian Statistics Agency, 2014 (van Schalkwyk, 2016).
Approximately 90% of wildlife occurs outside formally proclaimed protected areas. Only some 5% of game is found in national parks and protected areas. More than 80% of larger plains game species, including kudu, are found on privately owned farms, which comprise about 44% of the surface area of the country (van Schalkwyk, 2016). Wildlife numbers are projected to rise to about 5 million head over the next 50 years as wildlife or game farming is becoming an increasingly important economic activity, while land under domestic livestock is projected to decrease (van Schalkwyk, 2016).

It is predicted that southern Africa can expect an increase in temperature, with the maximum temperature increase of 2 – 6 degrees Celsius in the interior, with a concurrent 10% decrease in average annual rainfall in the northern and southern regions and a 20%

decrease in the central areas, over the next 30 years. This will negatively impact on livestock farming, whereas well-adapted game species will play an increasingly more important economic role as a replacement (van Schalkwyk, 2016).

In the following short sections, a number of concepts and terms will be briefly illustrated as they relate directly to the subsequent parts of this dissertation and will be referred to repeatedly in the text.

Veterinary Cordon Fences

Contagious bovine pleuropneumonia and the outbreak of Rinderpest in 1897 necessitated a greatly expanded veterinary infrastructure and the first veterinary laboratory was erected at Gammams near Windhoek in 1897. To prevent the spread of Rinderpest, a veterinary cordon line was established, which was the very beginning of the Veterinary Cordon Fence (VCF) as it is known today (Schneider, 1977).

VCFs are game-proof and stock-proof fences, which were erected inside Namibia as well as on the borders with Botswana and Angola by the veterinary authorities in response to the Foot-and-Mouth Disease (FMD) outbreak during 1961 to 1963 (Figure 1). The fences run from North to South and from East to West, effectively dividing the country into compartments. In some parts, they consist of a combination of game-proof and stock-proof fences. They are maintained by the Directorate of Veterinary Services since 1963 as part of the FMD control and containment protocol in Namibia (Anon., 2011). The fence on the border with Angola, stretching from the Kunene River in the west to the Okavango River in the east, has been extensively vandalized to the extent that it has disappeared completely, but there are plans to reconstruct the fence, without which it is impossible to prevent uncontrolled movement of livestock between the two countries (Maseke, personal communication). The northern VCF is also known as the “Red Line”. No movement of cloven-hoofed animals and animal products is allowed across the “Red Line” from North to South (Anon., 2011). This effectively cuts off the animal population in the NCAs from the rest of Namibia. The majority of cattle are kept in the NCAs.

Figure 1: Double Veterinary Cordon Fence (Photo: DVS)

Bush Encroachment

Bush encroachment is a term generally used to describe the invasion of grassy rangeland areas by indigenous woody plants. Namibia is affected by this process on a very extensive scale. Bush encroachment currently affects about 260 000 sqkm of farmland in 8 of the country's 14 regions (Gschwender, 2017; Joubert et al., 2016). This amounts to roughly 30% of Namibia's surface area (Gschwender, 2017). Bush encroachment severely reduces biodiversity and the formation of groundwater. It lowers the productivity and livestock carrying capacity for grazers of pasture land by about two thirds. Species of indigenous woody plants that contribute to bush encroachment include *Acacia mellifera, Acacia reficiens, Acacia luederitzii, Acacia erubescens, Acacia fleckii, Acacia nilotica, Acacia tortillis, Colphospermum mopane, Dichrostacys cinerea, Terminalia prunioides, Terminalia sericea*, and *Grewia flava.* Widespread and excessive bush density seems to be the result of rangeland mismanagement (Joubert et al., 2016), or at least changes in rangeland management practices including the reduction of browsing species and the prevention of fires.
Meanwhile, the bush encroachment process, apart from favouring browsing animal species, has developed into a huge biomass resource, estimated at about 200 million tonnes. Measures to combat bush encroachment, known in Namibia as "de-bushing", are creating new opportunities for the Namibian economy through the use of this resource for electricity generation and value chain development (Joubert et al., 2016) in other sectors like charcoal production, livestock feeds, fire wood or building materials.

Namibia has established a national de-bushing program which supports the large-scale activities to fight bush encroachment (Joubert et al., 2016).

Since kudu antelope are predominantly browsers and only occasional grazers, bush encroachment is seen a major contributor to a marked increase of the kudu population, which preceded the initial outbreak of rabies in 1977.

Namibia is renowned for its vast wilderness settings and rich wildlife populations. However, prior to 1970 national wildlife populations were on a downward trend, and it was not until 1968, when freehold farmers were given limited rights of proprietorship over wildlife, that commercial farmers acquired incentives to manage their wildlife for gain. In 1975, these rights were reinforced through the promulgation of the Nature Conservation Ordinance of 1975, and since then wildlife numbers on commercial farmlands have increased by more than 80% (Barnes et al., 1996).

Conservancies

The term "Conservancy" has often been defined, and the following definition has been formulated by the Conservancies Association of Namibia (CANAM, 2018):
A Conservancy is a legally protected area of a group of bona fide land-occupiers practicing co-operative management based on:
(1) A sustainable utilization strategy,
(2) Promoting conservation of natural resources and wildlife,
(3) Striving to re-instate the original bio-diversity with the basic goal of sharing resources amongst all members (CANAM 2018).
The first conservancy on commercial farmland was established in 1991 (CANAM, 2018).

Communal conservancies are self-governing, democratic entities, run by their members, with fixed boundaries that are agreed with adjacent conservancies, communities or land owners. Conservancies are recognised by the Ministry of Environment and Tourism (MET), but not governed by the Ministry.

Namibia is one of a few countries in the world where habitat conservation and protection of natural resources is addressed in the constitution. Article 95 of the Namibian Constitution states: *"The State shall actively promote and maintain the welfare of the people by adopting, inter alia, policies aimed at the following: (l) maintenance of ecosystems, essential ecological processes and biological diversity of Namibia and utilization of living natural resources on a sustainable basis for the benefit of all Namibians, both present and future; in particular, the Government shall provide measures against the dumping or recycling of foreign nuclear and toxic waste on Namibian territory."* (The Constitution of the Republic of Namibia).
In 1996, the Government of Namibia introduced legislation giving communities the power to create their own conservancies. The legislation allowed local communities to create conservancies that managed and benefited from wildlife on communal land while allowing the local community to work with private companies to create and manage their own tourism market. Currently there are 82 communal conservancies in operation (Figure 2), in which the members are responsible for protecting and utilizing their own resources sustainably, particularly the wildlife populations for game hunting and ecotourism revenues. In addition to the communal conservancies, there are 24 conservancies that involve freehold farms.
(Figure 2).

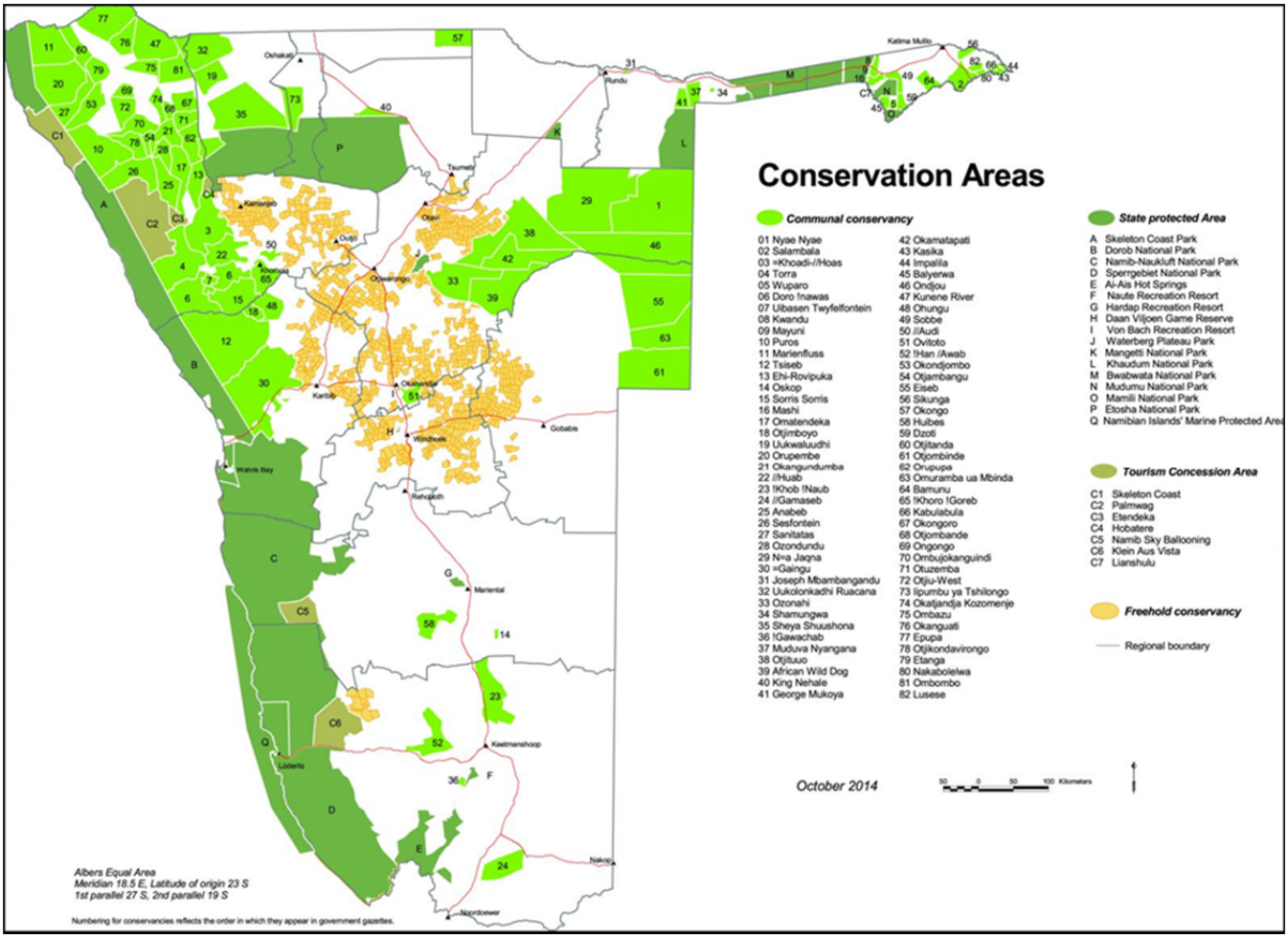

Figure 2: Map of Namibia with regions, state protected areas, communal conservancies, freehold conservancies and tourism concession areas

(Ministry of Environment and Tourism. MET, 2014)

2.2 The Greater Kudu: Distribution, Biology and Population

The Greater Kudu (*Tragelaphus strepsiceros*), a non-aggressive herbivore, is a large imposing and striking antelope, reaching 140 – 155 cm at the shoulder (Stuart, 1999). The Greater Kudu belongs to the order of *Artiodactyla* and to the *Bovidae* family. Male animals weigh on average 250 kg but can reach up to 315 kg (Estes, 1999) and females 180 kg (Stuart, 1999). In terms of body mass, it is third only to the eland, *Taurotragus oryx,* (700 – 900 kg) and the roan antelope *Hippotragus equinus* (220 – 300 kg). Its colour varies from reddish-brown to grey-brown with male animals – bulls – being darker in colour. The flanks are marked bilaterally by 6 to 10 very distinct vertical white stripes. There is a white stripe on the forehead between the eyes as well as a few white spots on the cheeks (Stuart, 1999). The ears are very large with a pink skin on the inside. The species is characterized by marked dimorphism since only bulls carry the distinctive spiralled horns. (Stuart, 1999). This antelope is an athletic jumper and can clear fences of up to 2 meters (Stuart, 1999). The Greater Kudu occurs mainly in the eastern and north-eastern parts of the southern African sub region, with a number of smaller populations in the South (Stuart, 1999). According to

the IUCN, the Greater Kudu occurs in Angola, Botswana, Central African Republic, Chad, Congo, The Democratic Republic of the Congo, Eritrea, Ethiopia, Kenya, Malawi, Mozambique, Namibia, South Africa, Swaziland, Tanzania, United Republic of Zambia and Zimbabwe. Estimates of the total population are around 482 000 with the largest number found in Namibia (East, 1999).

In Namibia, kudu occur very widespread with the greatest concentration in the central and north-central parts of the country (Figure 3) (Mendelsohn et al., 2009). In recent years, the range of the kudu population has extended into the more arid regions of the south-west as well as into the Kalahari in the east. A strong isolated population is found in the Karas mountains in the South. Kudu are characteristic animals for tree – and thorn bush savannah. Kudu do not occur in pure grassland or forests. In some parts they prefer rocky hills with acacia vegetation (Stuart, 1999). Kudu are almost exclusively browsers, utilizing a larger variety of different trees and shrubs than other antelope in the region. Occasionally kudu will utilize grass (Stuart, 1999). Bush serves both as food source and as protection. As browsers, kudu are not seen to compete with livestock (Owen-Smith, 1979; Owen-Smith et al., 1983; Owen-Smith et al., 1989).

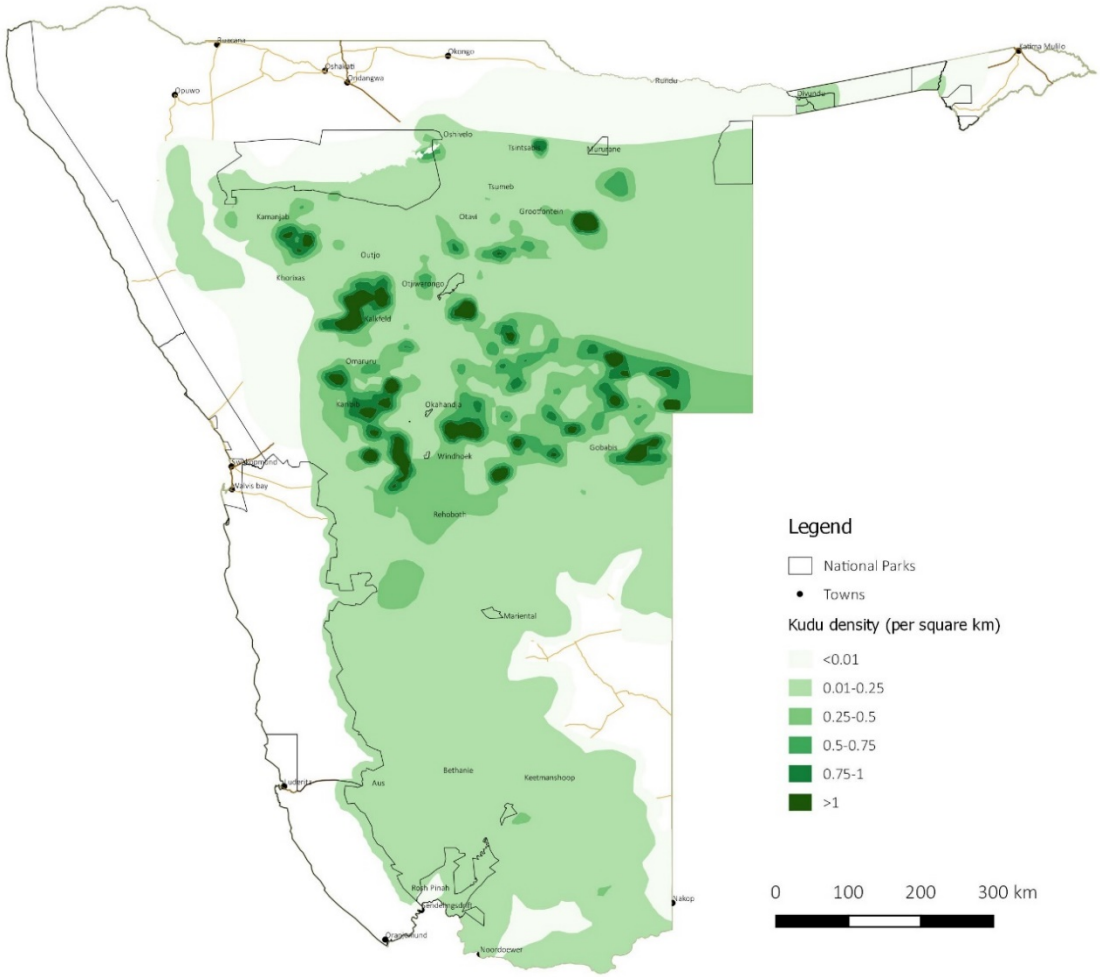

Figure 3: Map of Namibia with towns and national parks, indicating distribution and population density of the Greater Kudu (John Mendelsohn et al., 2009)

Kudu generally live in small family groups of between 3 to 10 animals, sometimes more, consisting of adult cows and their calves, young females and young bulls (Stuart, 1999) (Figure 4). During the mating season, which coincides with the cold dry season of the year, a mature bull will be found with these family groups (Stuart, 1999). Outside the rutting season, mature bulls are either found singly or in groups of varying numbers. After a gestation period of 270 days, cows calve from January until March (Stuart, 1999; Perrin et al., 1995). This time of the year coincides with the peak of the annual rainy season and should ensure optimum conditions for raising calves. The mating season from April until June is determined by decreasing daylight hours and the calving season coincides with increased rainfall (Perrin et al., 1995). These mating and calving seasons were confirmed by observations of animals used for experiments, which calved between January and March 2016 in the research facility. In Namibia, the Greater Kudu has adapted extremely well to the environmental changes characterized by bush encroachment, developed water infrastructure on commercial farms and a reduction in large predator numbers like lion brought on by commercial farming activities. It is thriving under these conditions and can be regarded quite frankly as a follower of civilization. Since it is predominantly a browser, it is not seen to be in competition with domestic cattle and sheep for grazing. In addition, the value of individual animals has increased due to trophy hunting activities.

Figure 4: Typical kudu family group (Photo: R. Hassel)

According to wildlife population estimates by Lindsey (Lindsey et al., 2013), the total population of kudu in Namibia is probably higher than previously reported (Barnes et al., 2009) and stands at 449 199, divided among the different regions as follows: Erongo 52 150, Hardap 60 962, Karas 32 970, Khomas 52 082, Kunene 54 756, Omaheke 41 093, Otjozondjupa 141 089 and Oshikoto/Oshana/Omusati 17 567, with an additional 2497 kudu in protected areas and 3595 on communal land (van Schalkwyk et al., 2016).

2.3 Aetiology of Rabies

2.3.1 Taxonomy of Lyssaviruses

Rabies (rabidus, Latin, = mad) is a highly fatal disease of warm-blooded vertebrates, including humans, due to an encephalitis or meningoencephalitis caused by a virus that is present in the saliva late in the infection. Rabies is usually transmitted by the bite of infected animals, most commonly dogs and other carnivores. The virus belongs to the genus *Lyssavirus* of the family *Rhabdoviridae* of the order *Mononegavirales* (Amarasinghe et al., 2018). The order *Mononegavirales* was created in 1991 to accommodate related viruses with nonsegmented, linear negative sense RNA genomes (Afshar, 1979). The family *Rhabdoviridae* (rhabdos, Greek, = rod) includes the genus *Lyssavirus*, the genus *Ephemerovirus* (named for bovine ephemeral fever virus), and the genus *Vesiculovirus* (named for vesicular stomatitis virus) plus a genus of fish viruses, two genera of plant viruses and a number of unassigned viruses (Swanepoel, 2004).

To accommodate the growing variety of “rabies-related” viruses, the genus Lyssavirus was established under the auspices of the International Committee on the Taxonomy of Viruses (ICTV). The name of the genus was derived from Greek mythology: Lyssa was a goddess or spirit of rage, fury, raging madness and frenzy (WHO, 2018). Most recently, the nomenclature of the *Mononegavirales* including the genus *Lyssavirus* was updated to accommodate a binomial species nomenclature (Afonso et al., 2016). Currently, the genus *Lyssavirus* comprises 16 recognized *Lyssavirus* species (Amarasinghe et al., 2018). These species, together with their hosts and worldwide distribution are summarized in Table 1.

Table 1: Host spectrum and distribution within the genus lyssavirus (adapted from WHO, 2018, and Amarasinghe et al., 2018)

Virus species	Virus	Abbreviation	Host Spectrum	Distribution
Rabies lyssavirus	Rabies virus	RABV	Wild and domestic animals, haemato- and insectivorous bats (North and South America), humans	Europe, Asia, America, Africa
European bat 1 lyssavirus	European bat lyssavirus 1	EBLV-1	Insectivorous bats (*Eptesicus serotinus*, *E. isabellinus*)	Europe
European bat 2 lyssavirus	European bat lyssavirus 2	EBLV-2	Insectivorous bats (*Myotis daubentoni, M. dasyscneme*)	
Bokeloh bat lyssavirus	Bokeloh bat lyssavirus	BBLV	*Myotis nattereri*	
Lleida bat lyssavirus	Lleida bat lyssavirus	LLEBV	Isolated from *Miniopterus schreibersi* (Iberian Peninsula)	
West Caucasian bat lyssavirus	West Caucasian bat virus	WCBV	Isolated from *Miniopterus schreibersi* (Kaukasusregion)	
Kotalahti bat lyssavirus	Kotalahti bat lyssavirus	KBLV[1]	Detected in *Myotis brandtii* (Finland)	
Lagos bat virus	Lagos bat virus	LBV	Fruit bats (Megachiroptera)	Africa
Mokola lyssavirus	Mokola virus	MOKV	Isolated from shrews, rodents, humans, dogs, cats, shrews	
Duvenhage lyssavirus	Duvenhage virus	DUVV	Insectivorous bats	
Shimoni bat lyssavirus	Shimoni bat virus	SHIBV	Isolated from *Hipposideros commersoni*	
Ikoma lyssavirus	Ikoma virus	IKOV	Isolated from civet cat*, Civettictis civetta*	
Australian bat lyssavirus	Australian bat lyssavirus	ABLV	Flying foxes and Insectivorous bats(Mega/Micro-chiroptera)	Australia
Aravan lyssavirus	Aravan virus	ARAV	Isolated from *Myotis blythi*	Asia
Khujand lyssavirus	Khujand virus	KHUV	Isolated from *Myotis mystacinus*	
Irkut lyssavirus	Irkut virus	IRKV	Isolated from *Murina leucogaster*	
Gannoruwa bat lyssavirus	Gannoruwa bat lyssavirus	GBLV	Isolated from *Pteropus medius* (Sri Lanka)	
	Taiwanese bat lyssavirus	TWBLV[2]	Isoliert from *Pipistrellus javanicus* (Taiwan)	

[1] only viral RNA found (Nokireki et al., 2018), not yet proposed as distinct species
[2] Hu et al., 2018

Bats have been identified as important reservoirs of zoonotic viral pathogens including rabies (Calisher et al., 2006; Hayman, 2016; Hayman et al., 2012). Rabies constitutes one of the most important viral zoonoses and poses a significant threat to public health globally. While RABV appears to be restricted to bats of the New World, related lyssaviruses have been detected in bat populations across Africa, Eurasia and Australia (Banyard et al., 2011, 2014). In fact, bats have been identified as the reservoir hosts for all lyssaviruses, except Mokola virus and Ikoma virus. For the latter two viruses the reservoir host remains to be determined (Banyard et al., 2011; Banyard et al., 2014).

Phylogenetic analyses suggest that lyssaviruses developed in bats and that host switching to carnivores occurred approximately 1 000 years ago (Badrane et al., 2001). Particularly the time of divergence has been controversially discussed and a co-evolution of bats and lyssaviruses and a dispersal in parallel to the splitting of continents was proposed (Rupprecht et al., 2017).

2.3.2 Lyssavirus Morphology

Lyssavirus particles are bullet-shaped, approximately 180 nm long and 75nm wide. They consist of a nucleocapsid of 160 x 50 nm, which is surrounded by a bilayer lipid envelope, derived from host cell membranes, through which flattened 10 nm spikes or peplomers, each composed of three molecules of glycoprotein (G protein), project over the entire surface of the virion, except at the blunt end (Dietzschold et al., 1978; Murphy,1975). Underlying the lipid membrane is a layer of membrane or matrix protein (M protein), which binds to the nucleocapsid protein (N protein) of the viral core and holds the envelope in place (Emerson, 1985). The rabies genome encodes five proteins: Nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and polymerase (L) (Finke & Conzelmann, 2005).

2.3.3 Transmission of Rabies

Lyssaviruses are primarily transmitted through the saliva of an infected animal, and infection occurs primarily via bite wounds. Potentially, the virus may also be transmitted if saliva of an infected animal enters an open skin cut or wound or mucous membrane, such as those in the mouth, nasal cavity or eyes. Saliva becomes infectious a few days prior to the onset of clinical signs (Fooks et al., 2017). Albeit rare, the transmission of rabies following non-bite exposure, particularly via ingestion or via aerosols was also documented (Afshar, 1979). The latter mode of transmission was speculated to be responsible for the occurrence of human rabies deaths after a non-bite encounter with bats in the Americas (Messenger et al., 2002, Warell et al., 1995).
Terrestrial (non-bat) rabies is mostly associated with mesocarnivores which act as reservoirs for the disease. While in the developing world, where more than 90% of human rabies cases occur (see Chapter 2.4.), the domestic dog is by far the most important rabies reservoir (WHO, 2018). Several wildlife species such as foxes, jackals, skunks, raccoons and mongoose can also serve as reservoirs. Infections of other non-carnivorous mammals usually terminate further transmission and lead to a dead-end (Mollentze et al., 2014). There are only few exceptions, where spill-over infections in non-carnivorous mammals have led to

onward transmissions, as opposed to a dead-end infection, one of which is presumably kudu rabies in Namibia (Scott et al., 2012).

2.4 Occurrence and Burden of Rabies

2.4.1 Rabies globally

Human rabies is present in 150 countries and territories and on all continents, except for Australia and Antarctica. Estimates indicate that globally human mortality due to endemic canine mediated rabies is highest in Asia with the highest incidence and mortalities reported in India. This is closely followed by Africa; however, no reliable data are available from large parts of the continent. Minimal information is also available regarding the scale of the burden of rabies in the Middle East and Central Asia (WHO, 2018).

Latin America and the Caribbean
This region has seen a substantial reduction in the number of human and animal rabies cases following the implementation of dog rabies control programs.
The most recent comprehensive estimate of the burden of rabies includes productivity losses due to mortality or morbidity expressed as "Disability Adjusted Life-Years" (DALYs), direct costs such as those of rabies vaccines and immunoglobulins, and indirect costs such as transport and loss of income incurred by patients. Livestock losses and the costs of surveillance and preventive measures such as dog vaccination are also included (Hampson et al., 2015).
The numbers of cases of human and dog rabies have decreased significantly in this region as a result of sustained control (Vigilato et al., 2013). Between 2013 and 2016, dog-mediated human rabies was reported only in Bolivia, Brazil, the Dominican Republic, Guatemala, Haiti, Honduras, Peru and Venezuela (WHO 2016, WHO 2018); see also Figure 6.

Asia
An estimated 35 000 human deaths (59.6% of global deaths) and loss of approximately 2.2 million DALYs occur per year in Asia due to dog-mediated rabies (Hampson et al., 2015). India accounts for the most deaths in Asia (59.9% of human rabies deaths) and globally (35% of human rabies deaths); see also Figure 6.

Africa
In Africa, an estimated 21 476 human deaths occur each year due to dog-mediated rabies (36.4% of global human deaths; see also Figure 6), with a loss of 1.34 million DALYs (Hampson et al., 2015). In one global cost study, Africa was estimated to spend the least on Post Exposure Prophylaxis (PEP) (3.28% of the global non-human mortality cost) and has the highest cost of human mortality (45%), indicating that many lives could be saved if access to PEP was improved or the prevalence of dog-mediated rabies reduced (Anderson 2015). Rabies monitoring is generally poor in Africa. Virtually all African countries have the required veterinary and medical infrastructures, but many have so far been unable to devote adequate resources to monitoring and controlling rabies in the face of poverty, prolonged droughts, other priorities or armed conflict (Fahrion et al., 2017; Addy, 1985; Ayalew, 1985; Ibrahim et al., 1985; Lawrence et al., 1980).

Central Asia and the Middle East

The disease burden due to dog-mediated rabies is estimated to be 1875 human deaths and 14 310 DALYs in Central Asia and 229 human deaths and 1875 DALYs per year in the Middle East (Hampson et al., 2015); see also Figure 6.

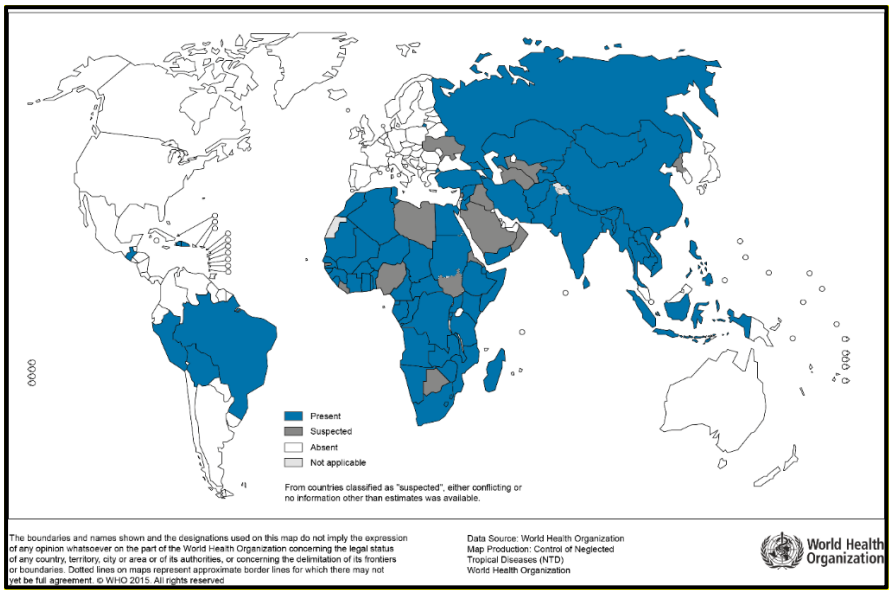

Figure 5: Occurrence of dog-transmitted rabies cases world-wide (WHO, 2014)

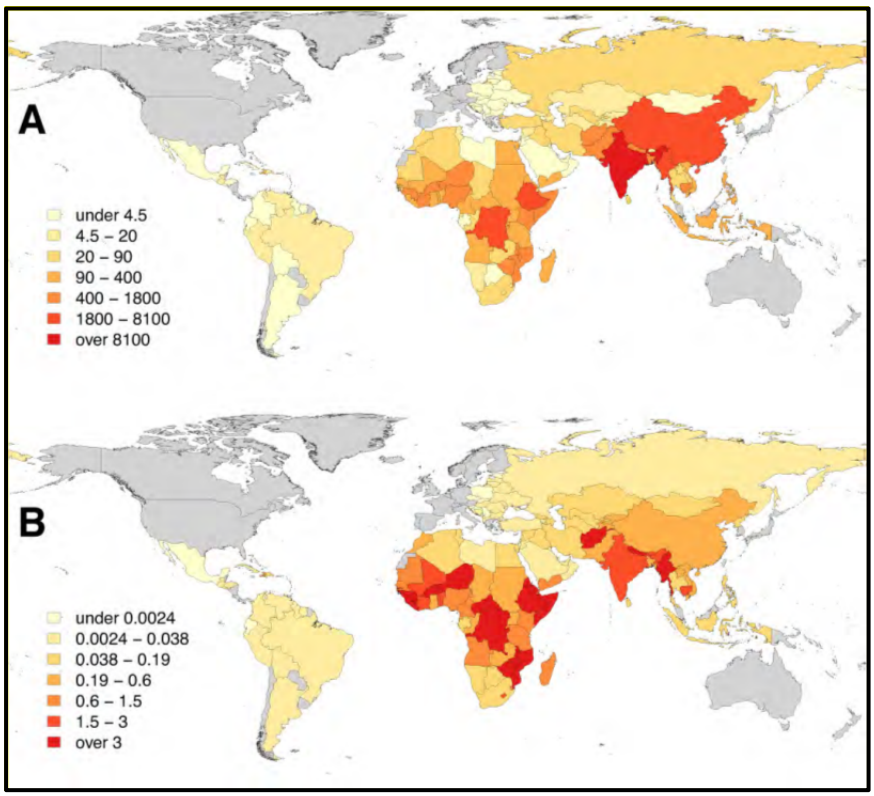

Figure 6: Global burden of dog-transmitted human rabies (WHO, 2018)

A: Human deaths from rabies per year; B: Death rates per capita per year (per 100 000 population); countries shaded in grey are free from canine rabies (Hampson et al., 2015)

2.4.2 Rabies in Africa

It is often quoted that human deaths due to rabies in Africa are second only to Asia. Estimates of burden have always been uncertain due to the absence of reliable data. In Africa, an estimated 21 476 human deaths occur each year due to dog-mediated rabies (36.4% of global human deaths), with a loss of 1.34 million DALYs (Hampson et al., 2015). According to the Executive Director of the Global Alliance for Rabies Control, L. Nel, rabies is a neglected disease all over Africa, and the continent suffers the highest per capita rate of human rabies deaths in the world (PARACON conference, July 2015).

In addition to RABV, the lyssaviruses circulating in Africa include Lagos bat virus, Mokola virus and Duvenhage virus (Swanepoel et al., 1993). A summary of lyssavirus species present in Africa as well as host species is contained in Table 1.

In Africa, the classical reservoir and vector of rabies virus is the domestic dog. It accounts for the majority – 99% - of human deaths, most of which involve children under the age of 15 years (Scott et al., 2016). Several other carnivore species like the black-backed jackal (*Canis mesomelas*) and the bat-eared fox (*Otocyon megalotis*) can maintain sylvatic cycles of rabies (Bingham, 2005). These wildlife vectors are not only able to contribute to disease maintenance, but also to the spread and cross-species transmission among various mammalian species, including spill-over hosts such as cattle and other livestock (Swanepoel et al., 1993, von Maltitz, 1950; Hübschle, 1988). In South Africa a distinct cycle of rabies is present in mongoose, which act as host, but only in certain areas (King et al.,1993; von Teichman et al., 1995; Nel et al., 2005).

2.4.3 Rabies in Southern Africa

In southern Africa, 4 species of the genus Lyssavirus, which cause rabies, are endemic. The most common is Rabies lyssavirus; RABV. Others are Lagos bat lyssavirus, LBV, Mokola lyssavirus, MOKV and Duvenhage lyssavirus, DUVV. Human infections are mostly due to the canine biotype of RABV (WHO/GARC, September 2014) (Table 1).

The following sections aim to provide a concise overview of the rabies situation in those countries in southern Africa with common borders with Namibia, since it is felt to be prudent to offer this comparison, in order to complete the picture regarding rabies in southern Africa.

South Africa

According to historical writings suspected rabies involving dogs and/or humans was observed in the Western Cape Province in 1772, 1825, 1826 and 1883, in KwaZulu-Natal Province in 1823 and 1857 and in the Free State Province in 1961 (Cluver, 1927; Henning, 1956; Neitz et al., 1932; Snyman, 1940). In 1893, an outbreak of the disease in dogs in the Eastern Cape Province was diagnosed by inoculation of rabbits and this was the first confirmed diagnosis of rabies on the African continent. Further investigation suggested that the first case had actually occurred in a dog imported from England in September 1892, which had become rabid a few weeks after arrival. Apparently, 90 dogs, 7 cats and a few cattle were affected (Eddington, 1895; Henning, 1956; Hutcheon, 1894). After 1894, rabies was not confirmed again for 34 years, but there was increasing anecdotal evidence of an

epidemic form of the disease associated with viverrids present in Eastern and Northern Cape and Free State (Swanepoel, 2004). The disease was eventually confirmed again in 1928 in two children bitten by a yellow mongoose in the Northwest Province (Herzenberg, 1928). Since that time, rabies has been diagnosed regularly in South Africa (Swanepoel, 2004). Mongoose rabies forms a distinct cycle in South Africa but is limited to certain areas (Meredith, 1982). Spread of canine rabies from Botswana into the Limpopo Province occurred in June 1950. From there it spread to the more densely populated areas to the east and entered Mozambique in 1952 (Swanepoel, 2004). Between 1928 and 2000, a total of 15 630 confirmed cases of rabies were recorded in animals, of which 9 777 were domestic and 5 853 wild animals, in addition to 441 cases in humans (Swanepoel, 2004).
Rabies is a notifiable disease in South Africa in terms of Regulation 328 of the Animal Health Act of 1977.

Each year confirmed human rabies cases are recorded in South Africa. In the past years, the majority of confirmed human rabies cases have occurred in the provinces of KwaZulu-Natal, Eastern Cape and Limpopo, mainly amongst children under the age of 10 and up to 92% are due to rabid dog bites. Consequently, people, especially children, living in provinces, where dog rabies is wide spread, are most at risk of contracting the disease. This accounts for about half of the population of South Africa. In 2012, there were 12 laboratory confirmed human rabies cases, which was twice the number reported in the previous year.

At least 600 to 700 cases of rabies are diagnosed in domestic and wild animals annually. Mostly dogs, but also cattle and the yellow mongoose, account for approximately 85% of all confirmed cases. Additionally, the black-backed jackal and the bat-eared fox are vectors of the disease. In 2012, a total of 834 cases of animal rabies were laboratory-confirmed, of which 508 were dogs, 212 other domestic animals and 114 wildlife. This is a steep increase from what had been reported in 2011, with a total 503 cases, 331 dogs, 105 other domestic animals and 67 wildlife (WHO, 2014).

The current dog rabies epidemic that is persisting in parts of South Africa since 1987 is believed to have originated from dog rabies-endemic areas in southern Mozambique (WHO/GARC, September 2014).

Angola

Rabies is a notifiable disease in Angola. However, it is not known whether the Laboratório Regional de Veterinária de Luanda, or any other laboratory carries out rabies diagnostics. It is not clear whether the human rabies cases reported to the OIE are laboratory confirmed, or diagnosed on clinical grounds only.
The main vector for rabies in Angola is the domestic dog. In the first half of 2012, 5 dogs were reported to have died from rabies. As a response, 128 dogs were vaccinated. In 2011, a total of 30 dogs died of rabies and subsequently 3502 animals were vaccinated. For the same year the number of dogs that have received routine vaccination was 330,866. Rabies appears to be present in wildlife, but there is no rabies surveillance of wildlife in Angola (WHO, 2013).

Botswana

On average, less than 200 cases of rabies are diagnosed in domestic and wild animals in Botswana annually, the majority of cases being recorded in the eastern part of the country. The most common animals affected are cattle, goats and dogs. In wildlife, it is by far most common in jackal. In recent years, there has been a shift to larger numbers of infected dogs being reported than rabid cattle and goats. According to the data submitted to SEARG in 2012, a total of 71 cases of animal rabies were laboratory confirmed, of which 50 were dogs, 21 other domestic animals and 0 wildlife (WHO/GARC, September 2014).

Zambia

Rabies is a notifiable disease in Zambia in terms of the Animal Health Act 27 of 2010 and the Public Health Act. Rabies in humans is mostly diagnosed on clinical signs only. One human case was laboratory confirmed in 2009. According to SEARG data, 3 human cases diagnosed on clinical grounds alone, were reported in 2011 and 5 in 2012. The main source of exposure of humans to rabies in Zambia are rabid domestic dogs and wild foxes.
The most common animal reported to be rabies-positive is the domestic dog. In 2011, 36 cases and in 2012, 24 cases were confirmed in dogs, while other domestic animals accounted for 5 and 3 cases in 2011 and 2012, respectively. One positive wildlife case each was reported in 2011 and 2012 (WHO/GARC, September 2014).

Zimbabwe

Rabies is a notifiable disease in Zimbabwe. Animal rabies is regulated by three different national policies. Rabies in humans in Zimbabwe is mostly transmitted through the bite of an infected domestic dog. According to data submitted to SEARG, there were 6 human laboratory confirmed cases in 2011. In 2012 only 2 human cases were confirmed, while 11 959 people were reportedly bitten by dogs.

In Zimbabwe, the domestic dog accounts for about 45% of all animal rabies cases. 71.3% of Zimbabwe's dog population is located in rural areas, thus, urban dog rabies is generally not a problem, except in the region of Mutare in eastern Zimbabwe. In 2011, a total of 134 dogs, 65 other domestic animals and 18 specimens of wildlife tested positive for rabies. In 2012, there were 59 confirmed dog rabies cases, 57 cases in other domestic animals and 20 cases in wildlife, most commonly the side-striped jackal (*Canis adustus*) and the black backed jackal (*Canis mesomelas*). Data reported to the OIE World Animal Health Information System for 2011 are not consistent with data reported to SEARG (WHO/GARC, September 2014).

2.5 Rabies in Namibia

2.5.1 History

Namibia belongs to the limited number of countries worldwide, where both dog rabies as well wildlife rabies cycles prevail.
At the turn of the 19th to the 20th century, rabies was virtually unknown in Namibia and there is only one reference according to which the disease may have occurred prior to 1900 (Schneider, 1977). The year 1887 is referred to by the Herero people as “Otjorundumba”, which can be translated as the “year of rabies”. It is reported that during that year significant numbers of cattle and small stock died as a result of bites from rabid dogs (Schneider, 1977). In June 1906, a case of rabies was confirmed in a dog in the town of Swakopmund. The origin of this dog, which bit two people, is unknown. In February 1926 rabies was reported in dogs in the area then known as Ovamboland, but not confirmed. In September of the same year a medical doctor of the Finnish Mission reported that the disease was already known to the local population for the last 20 years and that the number of cases had increased over the years (Schneider, 1977). Only unspecific reports were received during the following years until 1935 when it was suspected that the disease was occurring in Kavango. According to a report by the district surgeon of Kavango, a suspected case of rabies had occurred in a hyena and 18 clinical cases of hydrophobia had been diagnosed in humans. Inhabitants of the Northern Communal Areas (NCAs) mentioned that, apart from dogs, the disease also occurred in cattle, goats, donkeys and pigs. Another case was confirmed in a dog in 1938, the first since 1906. The next case was confirmed in 1947 also in a dog in Rundu, Kavango (Schneider, 1977).

Until 1947 rabies only occurred in the NCAs. From 1948 onwards, rabies rapidly and extensively spread southward and occurred in cattle in the Outjo area. From there, the spread involved black-backed jackal (*Canis mesomelas)* and cattle reaching the Otjiwarongo area in 1949, the central districts of Gobabis and Windhoek in 1951, Keetmanshoop in 1952 and Karasburg in 1962. From 1975 onwards, rabies has been regarded as endemic throughout Namibia (Schneider, 1977) (Figure 7, Tables 2 and 3) and cases increased progressively from 1978 until 2016. Until 1975 rabies in domestic animals accounted by far for the greatest percentage of cases and wildlife rabies occurred almost exclusively in jackal (Table 2). This situation changed with the advent of rabies in kudu and other antelopes (Courtin et al., 2000).
It should be mentioned here that von Maltitz (1950) had postulated that oral transmission occurred in cattle in order to explain the high incidence of the disease on some farms when rabies spread southwards in Namibia at the end of the 1940’s (von Maltitz, 1950).

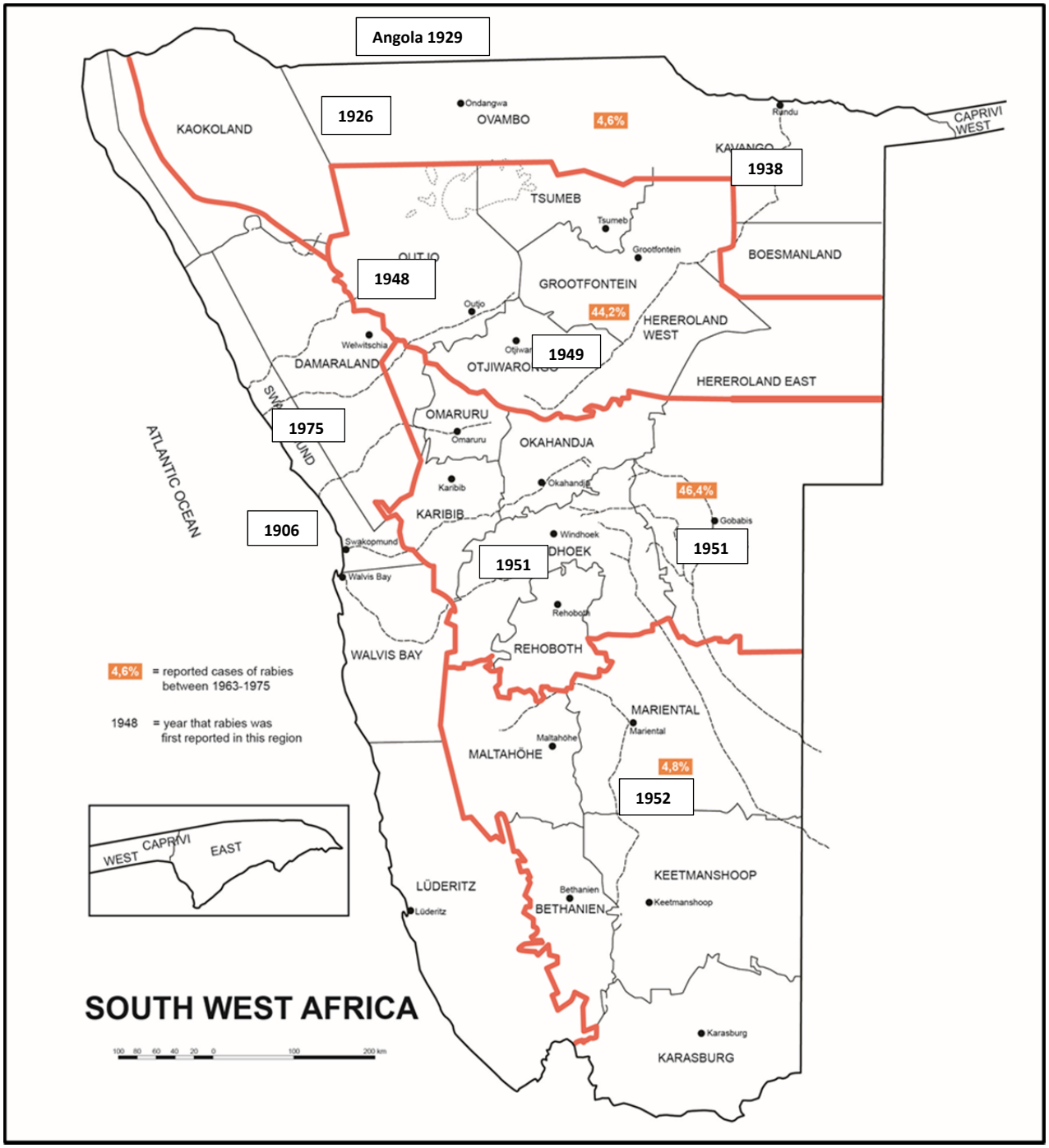

Figure 7: Map of Namibia showing districts, percentage of all rabies cases 1963 – 1975 and year of first occurrence of rabies (adapted from Schneider, 1977 and 1985)

Table 2: Rabies cases in Namibia in different animal species as percentage of total cases (Schneider, 1977)

1948 - 1956		1968 - 1975	
Cattle	64%	Cattle	49.8%
Dogs	20%	Dogs	17.5%
Jackal	9.4%	Jackal	15.2%
Felids	4.0%	Felids	4.3%
Viverridae	1.3%	Viverridae	3.5%
Sheep	1.3%	Sheep	1.8%
Goats	0	Goats	1.4%
Horses	0	Horses	1.0%
Other	0	Other	5.5%

From the early days onwards, rabies remained a problem in Namibia with livestock, dog and human cases recorded mainly in the NCAs, north of the VCF, where the human and livestock populations are the densest, jackal, kudu and cattle rabies occurring in the central ranching area south of the VCF and sporadic cases in felids and viverrids in the small stock ranching areas of the south of the country. Recent severe outbreaks of rabies in kudu have also been reported from the south of Namibia. Numbers of confirmed rabies cases have shown a steady increase over the 17-year period from 2001 – 2017 (Table 21).
In terms of the percentage of total rabies cases for the period 1963 – 1975, the distribution was as follows: Northern Districts 44.2%, Central Districts 46.4%, Southern Districts 4.8%, Northern Border Districts 4.6%.

Table 3: Rabies cases in districts of Namibia 1963 – 1975 (Schneider, 1977)

District	Year and number of reported rabies cases												
	63/64	64/65	65/66	66/67	67/68	68/69	69/70	70/71	71/72	72/73	73/74	74/75	Total
Gfn	27	19	12	8	6	23	4	1	5	15	12	4	116
Out Eto	3	4	2	4	9	14	6	2	5	23	9	0	81
Otji	4	15	12	7	3	9	2	-	3	8	8	8	79
Tsu	-	-	5	1	-	-	-	3	14	2	-	-	25
Ota	20	15	15	2	2	2	-	1	-	32	-	-	89
Total	54	53	46	22	14	48	12	7	27	80	29	12	
Total Northern Districts													**390**
Oma	1	1	18	2	4	2	-	5	9	1	2	3	48
Kari	-	-	7	-	-	1	-	2	11	-	4	0	25
Oka	2	35	16	4	4	4	10	11	14	13	10	8	131
Whk	1	7	5	5	2	8	3	9	11	3	4	5	63
Reho	-	1	-	-	-	1	-	-	-	-	-	-	2
Goba	8	7	8	1	4	30	5	6	9	35	18	10	141
Total	12	51	54	12	14	46	18	33	54	52	38	26	
Total Central Districts													**410**
Mar	1	2	-	1	-	10	3	1	-	3	-	1	22
Keet	-	-	-	-	-	-	2	2	-	2	3	4	13
Karas	-	-	-	-	-	-	3	-	-	1	-	-	4
Malta	-	-	-	-	-	3	-	-	-	-	-	-	3
Total	1	2	-	1	-	13	8	3	-	6	3	5	
Total Southern Districts													**42**
Ovam	-	-	-	2	-	4	5	8	3	7	-	1	30
Kaoko	-	-	-	-	-	-	-	5	-	-	-	1	6
Kavan	-	-	-	-	-	3	2	-	-	-	-	-	5
Total	-	-	-	2	-	7	7	13	3	7	-	2	
Total Northern Border Districts													**41**

2.5.2 Transmission: Vectors and Victims

The main vectors involved in the transmission of rabies in Namibia are dogs (Hassel, 1982; Schneider, 1985; Scott et al., 2016) in the NCA's and larger towns with cattle, cats and humans as main victims.

Jackal, mainly black- backed jackal (*Canis mesomelas)* in the northern and central areas of Namibia south of the VCF, transmit rabies mainly to cattle and less commonly to other domestic livestock, other wildlife and very rarely to humans (Hassel, 1982; Schneider, 1985; Scott et al., 2016).

In the south of Namibia rabies occurs only very sporadically, transmitted to sheep and goats by wild felids, viverrids and bat eared foxes (*Otocyon megalotis).* There is no evidence of a separate mongoose rabies cycle in Namibia. This is supported by the extremely small number of confirmed rabies cases in mongoose from 1977 – 2017 (Tables 19, 20 and 21).

Data on confirmed rabies cases are somewhat contradictory. For the purpose of this study, the data contained in the annual compilations of the Epidemiology Section of DVS are used (Tables 19, 20 and 21). The total number of confirmed rabies cases since the start of the first epidemic in kudu are summarised in Table 4.

Table 4: Summary of confirmed rabies cases for the period 1977 – 2017 (Directorate of Veterinary Services, Tables 19, 20 and 21)

Domestic Animals			Wildlife	
Cattle	2344		Kudu	1065
Dogs	1911		Jackal	536
Goats	405		Eland	65
Sheep	77		Bat-eared fox	78
Cats	236		Honey badger	29
Equines	77			
Other	38		Other	85

As far as wildlife rabies is concerned, a large number of different species have been affected by rabies over the years, with the largest numbers recorded in kudu and jackal (Table 4). Cases in bat-eared foxes (*Otocyon megalotis*), honey badgers (*Mellivora capensis*) and eland (*Taurotragus oryx*) are recorded regularly (Table 4). Less commonly rabies is confirmed in suricates (*Suricata suricatta*), mongoose species, wild cats (*Felis lybica)* and brown hyaenas (*Hyaena brunnea*). Only very few or just single cases have ever been recorded in cheetah (*Acinonyx jubatus*), leopard (*Panthera pardus)*, lion (*Panthera leo*), caracal (*Felis caracal*), genet (*Genetta genetta*), wild dogs (*Lycaon pictus)*, black rhino (*Diceros* bicornis), giraffe (*Giraffa camelopardalis*), wildebeest (*Connochaetes taurinus*), oryx (*Oryx gazella*), red hartebeest (*Alcelaphus buselaphus*), squirrel (*Cerus inauris)*, baboon (*Papio cynocephalus ursinus*), rock hyrax (*Procavia capensis*), waterbuck (*Kobus ellipsiprymnus*), roan (*Hippotragus equinus*), nyala (*Tragelaphus angasii*), steenbuck (*Raphicerus campestris*), dik-dik (*Madoqua kirkii*) and duiker (*Sylvicapra grimmia*) (DVS Tables 19, 20 and 21).

Human Rabies in Namibia

Rabies in humans in Namibia is mostly transmitted through rabid dog bites and occurs mainly in the northern parts of the country. Children up to the age of 16 are most affected.
Rabies cases in humans are predominantly reported from the NCAs, with Kavango, Oshana and Ohangwena regions having the highest case numbers. In contrast, human rabies cases in central and southern Namibia appear to occur sporadically. With 2.4 cases per 100,000 inhabitants and year on average, the rabies incidence was highest for Kavango region, followed by Oshana and Ohangwena regions. Rabies cases have been above 16 cases per year from 2011 till 2015 with the highest peak of 23 cases observed in 2015. The annual numbers of the two recent reporting years are below the previous average of reported cases. Of the total number of 113 cases, 62% of affected humans were male, and the majority (67%) were children and teenagers below 16 years of age (Hikufe et al., 2018 in press).
The expenditure on post exposure prophylaxis (PEP) treatment is currently 29 600 US Dollar annually (Scott et al., 2016).
The economic burden of rabies in kudu by far exceeds that posed by rabies in humans and in livestock in Namibia. By coupling the financial burden posed by rabies in kudu with that in the human population and in livestock, the total burden of rabies in Namibia is estimated to be USD 98 977 383 each year, which equals to USD 4.26 per capita (Scott et al.,2016).

Phylogenetic studies

In 1981, 2 brain samples of rabid kudu were submitted to the Pasteur Institute in Paris, France. Brain smears of each sample were tested with the usual panel of 18 monoclonal antibodies (anti-nucleocapsid antibodies). The only difference between the kudu strains and all other African strains tested previously appeared to be the 17th monoclonal antibody of the panel (Table 25) (Hassel, unpublished data).

A panel of 37 rabies virus isolates was collected and studied by Mansfield and co-workers. These isolates originated mainly from the northern and central regions of Namibia, between 1980 and 2003. The results of these examinations demonstrated a high degree of genetic similarity with respect to a 400 bp region of the nucleoprotein gene, with the virus isolates from kudu antelope (n=10) sharing 97.2 – 100% similarity with jackal isolates, and 97 – 100% similarity with those isolated from domestic dogs. Phylogenetic analysis suggested that these viruses were all of the canid rabies biotype of southern Africa. The viruses from kudu were closely associated with jackal isolates (n=6), bat-eared fox isolates (n=2) and domestic dog isolates (n=2) at the genetic level and identical at the amino acid level, irrespective of the year of isolation. These data suggest that jackal and kudu may form part of the same epidemiological cycle of rabies in Namibian wildlife and might demonstrate the close relationship between rabies virus strains that circulate within Namibia and those that circulate between Namibia and its neighbouring countries such as Botswana and South Africa. The molecular sequence and phylogenetic analysis of the Namibian rabies virus isolates described here, has demonstrated a high degree of similarity between isolates originating from different species, emphasising clearly the ease of transfer of RABV throughout Namibian wildlife, and between wildlife and domesticated species (Mansfield et al., 2006).

The existence of two epidemiological cycles of the canid biotype of RABV in southern Africa is well documented, an urban (canid) cycle within the domestic dog population, and a wildlife (or sylvatic) cycle which occurs among the jackal, kudu and bat-eared fox populations. Interestingly, the isolates from mongooses (*Viverridae*) have been shown to be phylogenetically distinct from the canid biotype (Mansfield et al., 2006).

Following large scale outbreaks of rabies in kudu in 1977, Scott and co-workers investigated the possibility of horizontal transmission of RABV in this antelope species. Through phylogenetic analyses, they aimed to demonstrate that rabies was being maintained independently within the Namibian kudu population. They also tested, through complete genome sequencing of four rabies virus isolates from jackal and kudu, whether specific mutations occurred in the virus genome due to host adaptation. They discovered a separate grouping of all rabies isolates from kudu as compared to those of any other canid species in Namibia, suggesting that rabies was being maintained independently in kudu. Additionally, they noted several mutations unique to isolates from kudu, suggesting that these mutations may be due to the adaptation of rabies to a new host. In their opinion they provided sound evidence that rabies is being maintained independently in the Namibian kudu population (Scott et al., 2013). These results contradict earlier findings of Mansfield and co-workers (Mansfield et al., 2006) and also recent phylogenetic work done by Müller and co-workers (unpublished), which suggest that jackal and kudu may form part of the same

epidemiological cycle of rabies in Namibian wildlife, although this cycle seems to differ from the traditional one, since rabies in kudu no longer appears to a dead-end infection.

2.5.3 Rabies in Kudu

It is generally accepted that the outbreak of rabies in kudu in the thorn bush savannah of Namibia in 1977 was preceded by a significant increase in the kudu population from 80 000 in 1972 to 200 000 animals (Hübschle, 1988), which is supported by the observation that game populations on commercial farmland increased by 80% (Chapter 2.1) (Barnes et al., 1996). The following factors contributed to this increase:

1. Bush encroachment. As kudu are predominantly browsers, the proliferation of woody plant species provides food to sustain large numbers of animals. Bush also provides cover and thus protection to these animals.
2. Development of water infrastructure on commercial farmland. Except during the rainy season, kudu are dependent on regular access to water sources. With the development of commercial farmland, the number of watering points for domestic livestock increased. Kudu are thus provided with an abundance of drinking water.
3. Reduction in the numbers of common predators praying on kudu, like lion.
4. The incorporation of kudu into the commercial farming activities as a trophy animal. Farmers were motivated to protect the species due to its increased economic value.

Symptoms of rabies in kudu

The most common clinical signs of rabies in kudu include:

1. *Loss of fear, tame appearance and uncharacteristic behaviour*. This is the most common and striking symptom of rabies in kudu. Affected animals lose all fear for humans and human dwellings, will not flee when they notice humans, will approach humans (Figure 8) and buildings and may even walk into buildings (Hassel, 1982; Barnard et al., 1981). Rabid kudu are commonly observed entering villages and towns and even the city of Windhoek, where they wander around aimlessly, become very agitated and pose a threat to humans and traffic. The handling of rabid kudu in urban areas including the safe destruction and disposal of the animals often presents a challenge to veterinarians, veterinary officials, other ministerial staff and law enforcement officers. Uncharacteristic behaviour can also include that affected animals are found in the company of domestic animals like cattle. Farmers have even reported observing rabid kudu licking cattle in the face (Hassel, 1982; Hassel, personal observations, unpublished data).

Figure 8: Rabid kudu approaching human

2. *Hypersalivation and frothing at the mouth*. This sign is observed in the majority of cases of rabid kudu. Single or multiple long strings of saliva drooling uninterruptedly from the mouth are a striking observation in affected animals. When fresh cadavers of kudu are found, it is quite common to see that the ground under the head is wet as a result of hypersalivation (Hassel, 1982; Figure 9).

Figure 9: Rabid kudu showing hypersalivation and frothing

3. *Various forms and degrees of paralysis.* In the advanced stage of the disease, different degrees of paralysis can be observed. These may include mild to severe ataxia, staggering gait, stiff gait, inability to clear fences, hindquarter paresis, recumbency and complete paralysis of the limbs (Figure 10). Lateral recumbency then precedes death (Hassel, 1982).

Figure 10: Paralysis of all four limbs in a rabid kudu

4. *Abnormal vocalization.* This can take the form of grunting or repeated bellowing while throwing back the head. This has specifically been observed in experimental cases (Hassel, 1982).

Less common signs include continuous tail wagging and tenesmus, weakness and listlessness, repeated licking of lips, continuous swallowing movements, biting or eating foreign objects, aggressive behaviour, walking in circles and into obstacles. Symptoms can occur singly, but in most cases a variable combination of symptoms is present, also dependent on the progression of the disease or the stage of the disease when it is observed (Hassel, 1982). Aggressive behaviour with attempted head butting has been witnessed on a number of occasions by the author.

Observations of signs in diseased kudu made by veterinarians and farmers and reported in various surveys, sample submission forms, disease report forms and personal communications, as well as personal observations by the author of a large number of clinical cases, both naturally occurring as well as experimentally induced, led to the compiling of a list of symptoms (Table 7).

The incubation period and duration of the complete course and progression of the clinical phase of natural infections are unknown. Once any of the above symptoms are observed, the course is usually acute and affected animals will die within 24 to 48 hours. If paralysis

has already set in, death usually follows within 12 to 24 hours. To prevent contamination or infection of other animals and humans, as well as due to animal welfare considerations, the disease is not allowed to run its course, but kudu suffering from clinical rabies or suspected cases are usually destroyed as soon as typical signs are observed. This contributes to the lack of knowledge regarding the course of the natural rabies infection in kudu. In experimentally induced infections, the course of the disease was usually more acute and animals usually succumbed within 24 – 36 hours after the symptoms appeared.

2.5.4 Rabies Control in Namibia

2.5.4.1 Legislation

The national legislative powers for the control of rabies are contained in the Namibian Animal Health Act 1 of 2011 and its supporting regulations, at municipal level in the Municipal Dog Tax Ordinance 13 of 1967, supported by Model Regulations for the control of dogs in Municipal areas as contained in Government Notice 131 of 1968. Currently, the Animal Health Act 1 of 2011 is not supported by regulations. Therefore, in the interim old legislation, the Animal Diseases and Parasites Act 13 of 1965 as amended and its supporting regulations remains in force. The drafting of new regulations is at an advanced stage and they are expected to be published in the Government Gazette in due course.

Rabies in dogs, cats and wild carnivores, cattle, sheep, goats, pigs, horses, donkeys and mules is a notifiable disease in Namibia in terms of subsection 1 of section 2 of the Animal Health Act 1 of 2011 (Anon., 2013). The whole territory of Namibia is rabies-control area. The competent authority in control of animal health, veterinary public health, animal movement and animal disease control is the Directorate of Veterinary Services (DVS), within the Ministry of Agriculture Water and Forestry (MAWF), led by the Chief Veterinary Officer (CVO). All cases or suspected cases in the prescribed animal species have to be reported to an official of DVS and are recorded on the applicable Disease Report Form (DRF) or Herd Health Form and submitted to the Epidemiology Section of DVS (Anon., 2011).

2.5.4.2 Surveillance and Reporting

In general, surveillance is aimed at demonstrating the absence of disease or infection, determining the presence or distribution of disease or infection, or detecting as early as possible exotic or emerging diseases. The type of surveillance applied depends on the outputs needed to support decision-making. Surveillance in wildlife presents challenges that may differ significantly from those in surveillance in domestic animals (OIE, 2017). As far as the veterinary surveillance system in Namibia is concerned, the DVS comprises of 21 state veterinary offices with 68 state veterinarians and 112 AHTs in 2016 (Namibian Veterinary Council, 2018). In addition, there are 19 mixed private veterinary practices in Namibia with a fluctuating number of around 40 private veterinary professionals and their trained veterinary para-professional staff. Furthermore, four wildlife practices with four specialized wildlife veterinarians cater for the wildlife industry in Namibia (Veterinary Association of Namibia). The third component of the surveillance system is the diagnostic component in the form of

two veterinary diagnostic laboratories (see section 2.5.4.5). The system of surveillance is initiated by the reporting of cases or suspected cases of animal diseases by veterinary professionals and para-professionals, irrespective whether in private practice or employed by DVS, and the submission of samples to the two laboratories. Upon confirmation of the diagnosis and dissemination of the results, the necessary action can then be taken (Anon., 2011).

2.5.4.3 Vaccination

The vaccination of all domestic dogs at an age between three and six months with an approved anti-rabies vaccine is compulsory throughout Namibia according to current legislation. After the initial vaccination, dogs must be revaccinated within twelve months and thereafter at least every three years. For transporting dogs across international borders – import as well as export – a veterinary health certificate and transport permit are required. For this purpose, dogs need to be vaccinated not longer than 12 months before departure. Puppies younger than 3 months born from vaccinated mothers may be imported into Namibia but have to be confined by the owner at their final destination and presented for vaccination as soon as they are 3 months old. Unvaccinated puppies younger than 3 months born from unvaccinated dams are not allowed to cross international borders, nor are unvaccinated adult dogs (Anon., 2011).

Rabies vaccinations for domestic dogs and cats are provided by all state veterinary offices free of charge throughout the year and by private veterinary practices on an ongoing basis for a fee. Dogs on farms are vaccinated by AHTs (veterinary stock inspection staff) of the DVS during their routine farm inspection visits. In the NCAs, state veterinary staff will vaccinate dogs against rabies at the same time when stock owners present their cattle for vaccination against FMD and CBPP (DVS Annual Reports). At irregular intervals, state veterinarians may implement ad hoc or special vaccination campaigns for dogs in their area or in certain towns, if they deem this to be necessary. During such campaigns, vaccine will be provided to private veterinarians to enable them to administer vaccinations free of charge for the duration of the campaign. Despite these regulations and attempted control measures, dog rabies still prevails in Namibia, characterized by cyclic fluctuations of the annual number of confirmed cases.

No other domestic animals are subject to compulsory rabies vaccination, but owners are greatly encouraged to have their domestic cats and cattle vaccinated against rabies.

The keeping of wild carnivores in captivity, both canids and felids, is subject to specific regulations prescribed by the Ministry of Environment and Tourism. Part of the requirements is an annual health check of these captive animals by a veterinarian and compulsory rabies vaccination (Nature Conservation Ordinance 4 of 1975, OG 3469).

Riding clubs and stables for sport horses require their owners to have their horses vaccinated against rabies annually, if they want to be members of such clubs or stable their horses at such establishments. In most cases, the rabies vaccinations are administered together with vaccinations against African horse sickness, tetanus and equine influenza (Hassel, unpublished).

2.5.4.4 Rabies Control Strategy

After realizing that the current measures to control or curb the occurrence of rabies were ineffective, which is reflected in the increase in the rabies cases since 2000, the Ministry of Agriculture, Water and Forestry, through the DVS, developed the Rabies Control Strategy (Directorate of Veterinary Services, 2015). This was done in collaboration with the Ministry of Health and Social Services and the Veterinary Association of Namibia and introduced in March 2015. The purpose of the strategy document is to provide a framework for managing rabies in Namibia.

At a workshop held in 2012 the main constraints with regard to rabies control in Namibia were identified as follows (Directorate of Veterinary Services, 2015):

1. Lack of policy, strategy and standard operational procedures
2. Outdated or inadequate legislation for rabies control
3. Lack of co-ordination of rabies control activities nationally
4. Inadequate planning of rabies control activities
5. Low vaccination coverage
6. Lack of control of stray dogs and cats
7. Insufficient data of dog populations
8. Poor public awareness
9. Inadequate surveillance and reporting of rabies cases
10. Lack of stakeholder involvement
11. Lack of research into the disease

The main areas which required strengthening were identified as follows:

1. Inadequate transport for rabies control activities
2. Insufficient facilities and equipment to maintain the cold chain for rabies vaccines
3. Insufficient manpower to carry out rabies control activities
4. Insufficient law enforcement
5. Inadequate monitoring and evaluation of rabies control activities
6. Inadequate quarantine facilities to isolate and monitor suspected rabies cases

The objectives of this strategy are therefore to address these constraints and shortcomings in order to minimise the impact of rabies on humans and animals in Namibia.

The strategy comprises the following:

1. Review and update of the legal provisions on rabies control and enforcement procedures
2. Providing guidelines and standard operating procedures for rabies control in Namibia
3. Establishing baseline information on dog populations and circulating virus types to support rabies control
4. Co-operation with regional bodies (SADC) in rabies control
5. Strengthening stakeholder participation
6. Designing an effective vaccination program to increase vaccination coverage
7. Assigning dedicated staff for rabies control, particularly in high risk areas
8. Providing training to staff involved in rabies diagnosis and control
9. Increasing institutional and public awareness with regard to rabies and other zoonotic diseases

10. Providing adequate infrastructure, transport and equipment
11. Managing and controlling animal populations, particularly dogs
12. Providing an effective monitoring and evaluation program for the activities

Subsection 3.3.2 of the Namibian Rabies Control Strategy deals specifically with conducting research, stating that epidemiological research needs to be undertaken to gain a deeper understanding of the disease in Namibia. Research should focus on, but not be limited to:

1. Identifying rabies strains circulating in Namibia
2. Assessing the efficacy of vaccines used in Namibia, including oral vaccines
3. Investigating the feasibility of using alternative vaccines to control rabies in animals, e.g. oral vaccines
4. Research into wildlife rabies. Research will focus on finding suitable ways of dealing with rabies in wildlife particularly kudu antelope and wild carnivores
5. Undertaking feasibility and cost-benefit assessments of control measures of rabies in Namibia. Research in this area will focus on identifying the most suitable, efficient and effective ways of controlling rabies in Namibia

2.5.4.5 Sample Submission and Laboratory Testing

Submission and testing of specimens for rabies diagnosis is done according to the standard operating procedures as contained in Appendix 4 of the Rabies Control Strategy (Directorate of Veterinary Services, 2015).

Rabies diagnosis is provided by the Central Veterinary Laboratory (CVL) of the DVS in the capital Windhoek. A second rabies diagnostic facility is the Regional Laboratory (RL) situated in Ondangwa in the Oshana Region of the NCAs. Samples submitted to the CVL must be accompanied by a Test Request/Sample Submission Form QUA FRM 99 together with disease report forms, such as the Herd Health Form and Residue Sampling Form.

In case of suspected rabies, human exposure must be indicated on the form. Brain specimens should include the hippocampus, cerebellum and medulla oblongata, but half or even the whole brain may be submitted. Depending on the time and distance of transport, specimens can be submitted to the CVL either fresh (refrigerated), frozen or preserved in glycerol-saline or 10% buffered formalin. The CVL will even accept whole heads for testing and extract the required tissue from them.

The standard routine test for rabies performed on the specimens is the Fluorescent Antibody Test (FAT) (OIE, 2018; Dean et al., 1996). It is also referred to as the Direct Fluorescent Antibody test (DFA).

In 2009, Namibia took part in a diagnostic proficiency and protocol harmonisation workshop at the OIE Rabies Reference Laboratory for southern Africa (Sabeta et al., 2011). This helped to ensure that the highest diagnostic standards can be maintained in Namibia. Diagnostic capabilities were further improved when DVS introduced the routine implementation of a new diagnostic assay, which is currently undergoing global validation and is implemented on a large scale (Rupprecht et al., 2018). This test is the Direct Rapid Immunohistochemical Test (DRIT), (OIE, 2018; Lembo et al., 2006) and it has been used in various studies (Coetzer et al., 2017a; Coetzer et al., 2017b; Coetzer et al., 2014; Lembo et al., 2006; Middel et al., 2017; Rupprecht et al., 2014).

The CVL is now also able to perform a Real Time Polymerase Chain Reaction tests in case of suspect negative or inconclusive FAT results.

3. Material and Methods

3.1 Epidemiology

3.1.1 Data Collection

3.1.1.1 Survey 1977-1980

A first disease prevalence survey was conducted in 1980 covering the period from 1977 until 1980. This was done by means of a questionnaire distributed by mail to farmers in the areas affected by rabies among kudu at that time. Information was requested on the following:

1. Farm name, farm identification number, location (district) and size in hectares
2. Estimate of the kudu population or game count
3. Kudu population dynamics (increase, decrease or static)
4. Jackal population dynamics at the time (increase, decrease or static)
5. Number of kudu carcasses found on the farm
6. Number of clinical cases observed in kudu
7. Sex distribution of kudu affected by rabies
8. Number of affected jackals (carcasses and clinical cases)
9. Symptoms observed by farmers
10. Number of laboratory confirmed cases

Diagnostic procedures

Specimens: Brain specimens of suspected cases of rabies in a variety of game and domestic animals collected by veterinarians, stock inspectors and farmers, together with case reports, were submitted to the Veterinary Research Institute, Onderstepoort, South Africa, for examination. One half of the brain was preserved in 50% aqueous glycerine for serology and virus isolation while the other half was preserved in 10% formalin for histological examination. A positive diagnosis was made when at least one of the methods gave a positive result. The most important parts of the brains that were used for analysis included the medulla oblongata, cerebellum and hippocampus. When rabies was confirmed on a farm or in an area, the diagnosis of further cases was usually based on clinical symptoms of affected animals (Barnard & Hassel, 1981).

Tests: The FAT (Dean et al., 1973) was used for diagnosis. Apart from this test, the identity of the virus was confirmed by virus neutralization tests in 3-week-old mice as described by Koprowski (Koprowski, 1973). The reaction of guinea pigs and mice inoculated with the virus isolated from kudu was also compared with the reactions provoked by the inoculation of RABV isolates from other species of animals from other parts of the country during routine diagnostic procedures (Barnard & Hassel, 1981).

3.1.1.2 Survey 2012 - 2015

A second survey was undertaken between 2012 and 2015, covering the period from 2009 until 2015. This time, the questionnaires were distributed by electronic mail. They were

provided to organizations like the Namibia Agricultural Union, Livestock Producers Organization, Namibia Professional Hunting Association, Conservancies Association of Namibia and farmers associations, who subsequently distributed them to their members. Due to multiple memberships, it was not possible to determine the return rate of the distributed questionnaires. The parameters evaluated included those of the previous survey. In addition, information was collected on game counts, the number of water points and game fences, supplementation provided to game as well as the number of rabies cases in any other species than kudu.

Data collection further included the number of rabies cases in all species confirmed by laboratory testing (FAT), as provided by the epidemiology section of DVS, as well as records provided by the CVL, and information on the spatial distribution.

3.2 Animal studies

3.2.1 First Transmission Studies

Animals: kudu for the studies (n=12) were captured in 1980 by mass capture in the Hardap game reserve, Hardap Region, and transported to the Daan Viljoen game reserve outside Windhoek, where they were kept in isolation in a quarantine facility. Both game reserves belonged to the former Department of Nature Conservation and Tourism (now Ministry of Environment and Tourism). Capturing and transporting of the animals was carried out by officials of the Department of Nature Conservation and Tourism. At the time of their capture, no cases of rabies in kudu had ever been reported in the Hardap game reserve, which is situated 230 km away from the facility where the experimental animals were kept later. The animals were fed on lucerne hay *(Medicago sativa*) and antelope cubes containing lucerne hay, field grass hay and ground maize, as well as browse cut in the veld provided by the Department of Nature Conservation. The animals were allowed to adapt for 2 months and observed daily, prior to the implementation of the experiments (Barnard et al., 1982).
Since this was a joint project of the Department of Nature Conservation and the Directorate of Veterinary Services, no separate permits were required at that time. Two animals had to be euthanized due to injury during the process of offloading at the quarantine facility.

Virus titration: The brains and the salivary glands collected from free-living kudu that had died of rabies were preserved in 50% aqueous glycerine. The saliva was preserved in Eagle's medium, containing 5% bovine serum, 500 international units of penicillin and 500 micrograms of streptomycin per millilitre, referred to as EMA. The specimens were submitted to the Veterinary Research Institute, Onderstepoort, South Africa, for virus titration in 3-week-old mice. The time between the collection of the specimens and titration varied from 4 – 16 days. Specimens were kept at room temperature during most of the period (Barnard et al., 1982).

Groups of 6 mice were injected intracerebrally (ic) with 0.03 ml of tenfold dilutions of organ suspensions in EMA. The mice were observed for 28 days and the presence of rabies in mice that died was confirmed by FAT (Barnard et al., 1982).

Infectivity of saliva: Four kudu in captivity were experimentally exposed to infection. About 10 ml of saliva was collected from a kudu with natural rabies, found in the vicinity of Windhoek. Of this roughly 2 ml each was instilled directly into the nasal and buccal cavities of the four kudu. Two bovines were also infected. Their mucous membranes appeared to be intact. The experimental animals were observed daily. Brain specimens of experimentally infected kudu that died following the infection were collected and subjected to FAT to confirm the diagnosis (Barnard et al., 1982).

Figure 11: Applying saliva from an animal suffering from natural clinical rabies to an immobilized captive experimental animal

Infectivity of High Egg Passage (HEP) Flury virus vaccine of chick embryo origin: Three kudu kept in captivity in Namibia and three cattle kept at the Veterinary Research Institute in Onderstepoort were exposed in the same way to the HEP Flury virus grown in BHK 21 cell cultures. The HEP vaccine was a rabies vaccine registered for use in cats and cattle, produced by the Veterinary Research Institute Onderstepoort, Republic of South Africa. The freeze-dried virus was reconstituted with 1 ml of EMA and applied to the buccal and nasal mucosae of the experimental animals. Each animal received approximately 2 x 10^6 mouse LD 50 of virus (Barnard et al., 1982).
An additional three kudu and three cattle were exposed to HEP Flury virus added to their drinking water after water had been withheld for 24 hours. Each animal was supplied with 2

litres of drinking water containing 3 x 10^6 mouse LD 50 of HEP Flury virus. This procedure was repeated after 30 days in case of the cattle only (Barnard et al., 1982).
The experimental animals exposed to the HEP Flury virus were bled 30, 60 and 90 days after exposure. The serum was tested for the presence of rabies virus neutralizing antibodies with the mouse neutralization test (MNT). The test was carried out according to the constant virus serum dilution technique. (Koprowski, 1973) The CVS strain of rabies, diluted to contain 100 – 300 mouse LD 50/0,03 ml, was used as antigen (Barnard et al., 1982.)

Susceptibility of mice: Groups of 3-week-old mice were starved for 24 hours and then allowed to consume infected kudu brain ad libidum in a period of 2 – 4 hours. They were then supplied with mouse pellets and a 1/50 suspension (weight/volume) of infected brain in drinking water. It was estimated that each mouse consumed 0,2 to 1 g of infected brain. The mice were observed for 28 days. Brains of mice that died were examined by FAT (Barnard et al., 1982).

3.2.2 Studies Related to the Current Kudu Rabies Research Project

3.2.2.1 Regulatory Considerations

All activities related to the recent study conducted between June 2015 and August 2016 were implemented in accordance with the relevant legislation. This study was conducted under general Permits 101631, 101835, 101825, 101826 issued by the Namibian Ministry of Environment and Tourism for (i) capturing, transport and keeping of game for commercial purposes and (ii) research in regard to problem animals under the Nature Conservation Ordinance 4 of 1975 as well as a research permit issued by the Namibian Ministry of Environment and Tourism (Permit No 1984/2014 and 2152/2016). The facility conformed to the requirements of MET for captive game. The facilities as well as the standard operating procedures for maintaining quarantine conditions were approved by the Directorate of Veterinary Services of MAWF. The animals were transported from the capture locations to the research facility in terms of Veterinary Movement Permits issued by DVS.

3.2.2.2 Animals

The kudu (n=46) for the study were all donated by farmers and conservancies. They were captured in 4 different locations, one farm in the Kalkfeld Conservancy, two farms in the Namatanga Conservancy and one farm in the Richtberg Conservancy and transported by truck to the holding facility on Okosongoro Safari Ranch (Figure 14) in the Omaruru district. No cases of rabies had been reported from any of the farms of origin or from the neighbouring farms during the preceding 12 months. As far as could be reasonably established, none of the kudu had been vaccinated against rabies by the farmers on the respective or adjacent farms. This was done to ensure that no test animal incubated the disease or that previously vaccinated animals entered the trials. 36 adult female and 4 young male kudu were initially captured. It was decided to use mainly female animals, since kudu bulls are much more difficult to transport, more difficult to handle, consume a lot more food

and tend to be aggressive towards other strange kudu, if confined together with them. Solitary confinement of adult kudu bulls can also be problematic.

Twelve kudu were delivered to the holding facility by a farmer of the Kalkfeld Conservancy. These animals were part of a mass capture operation on that farm to reduce the number of game due to prevailing drought conditions. Two animals were lost during the adaptation period by not adapting to the new diet. Since they were not chemically immobilized, no serum samples could be collected from these animals at the time of capture, but only later on the day the vaccine was administered. From all other animals, intravenous blood samples were collected to obtain serum on the day of capture (Figure 13).

The animals from the Namatanga and Richtberg Conservancies were individually immobilized from a helicopter, using darts containing a mixture of 8 – 10 mg thiafentanyl (10 mg/ml Thianil, Wildlife Pharmaceuticals, Rocky Drift, White River, South Africa) and 100 mg azaparone. An example of an immobilised kudu is shown in Figure 12. Immobilization was reversed by intravenous injection of 80 – 100 mg of naltrexone (50mg/ml Trexonil, Wildlife Pharmaceuticals). In addition to collecting blood samples (Figure 13), the animals were identified by the insertion of numbered ear tags as well as subcutaneous microchips. They were also dewormed using an injectable doramectin preparation at a dose of 1 ml per 50 kg body mass (Dectomax, Zoetis, Sandton, Johannesburg South Africa) and received a long-acting antibiotic Peni-LA Phenix at 1 ml per kg body mass (Virbac RSA, Halfway House, South Africa) , multivitamin Kyrovite B Co Super 7 ml per animal (Kyron Laboratories, Benrose, South Africa), metabolic stimulant Kyrophos Metabolic-V 10 ml per animal (Kyron Laboratories) and long-acting sedative, perphenazine at 80 mg per animal (100 mg/ml, Kyron Laboratories) injections, prior to transportation. Three animals were lost due to capture stress and one as a result of injury during transport.

Figure 12: Kudu destined for the study immobilized with a dart containing thiafentanyl and azaparone fired from helicopter

Figure 13: Collection of blood samples from kudu at the time of capture

Animals were transported by road in specialized game transport trucks to the holding facility, where the experimental work was conducted.

The facility was a large, extensive holding structure for game generally referred to as “boma” on the farm Okosongoro Safari Ranch, located in the Omaruru district, roughly 200 km north-west of Windhoek (Figure 14). This boma allowed animals to be kept and fed in separate groups in individual pens and made it possible to sort the animals easily and to observe them conveniently. (Figure 14, upper left and right panels). All procedures including immobilization, treatment, vaccination and sampling were conveniently and safely carried out inside the facility. No animals were lost during the performing of any of these procedures. A 3.2-meter-high game- and predator-proof fence was constructed around the boma to turn it into a quarantine facility (Figure 14, lower left panel). For easy identification the experimental animals were fitted with coloured ear tags in the right ear. These ear tags displayed the group number and the individual animal number (Figure 14, lower right panel).
Following an inspection, the facility was approved by DVS for the experimental work on live animals, including the administration of live RABV. The facility exceeded the minimum requirements of MET for the keeping of antelope in captivity. The kudu were allowed an adaptation period of 2 – 3 weeks before any procedures were performed on them. During this period 3 animals were lost due to digestive problems while adapting from a natural browse diet to the diet fed in the boma.
The diet of the animals in captivity consisted of lucerne hay (*Medicago sativa)* fed ad libidum, pelleted game feed ad libitum as well as acacia pods when available. Fresh feed was supplied daily and the individual pens were cleaned daily, and faeces and feed rests removed from the facility. These strictly implemented hygiene protocols were the key to very low external and internal parasite burdens. Fresh drinking water was provided ad libidum and replenished daily. Vitamin and mineral supplementation was provided via the drinking water at 3 -monthly intervals (Game Min Capture - Boma; Afrivet, Faerie Glen, South Africa) at 1 ml per litre of drinking water. The animals were dewormed using an injectable doramectin anthelmintic at 1 ml per 50 kg body mass (Dectomax Zoetis, South Africa) at roughly 4 monthly intervals when the animals were immobilized for other procedures. Worm burdens and coccidia infection were monitored by faecal examination of samples submitted to the CVL.

Test groups: For the first round of experiments, the captive animals were divided into different groups of four or five animals each of the same sex. The sizes of the individual groups were determined by the size of the pens and by the consideration to divide the total number of animals into groups of similar size that could be managed and handled easily, safely and conveniently. The groups were housed in separate pens (Table 5).

Table 5: Test groups

Group No.	Ear Tag	No. of Animals	Purpose	Subgroups
1	Orange	20	1. Control 2. Experimental transmission	4
2	Yellow	9	Intra-muscular vaccination	2
3	Green	10	Oral vaccination	3

Figure 14: The research facility (game boma) at Okosongoro Safari Ranch
clockwise from top left:
Aerial view of research facility (game boma) at Okosongoro Safari Ranch
Central passage between enclosures with overhead catwalk
Group of experimental animals inside the facility
Perimeter fence with sign board

For any manipulation, e.g. sampling, vaccination etc., the animals were always immobilized and sedated with a combination of 6 – 8 mg of thiafentanil oxalate (10 mg/ml Thianil, Wildlife Pharmaceuticals, Rocky Drift, White River, South Africa) and 100 mg azaparone (Kyron Laboratories, Benrose, South Africa) reversed with 80 mg naltrexone hydrochloride (50 mg/ml Trexonil, Wildlife Pharmaceuticals). Sedation was carried with a X-Caliber CO_2-operated dart projector with syringe darts of 2 ml with a 14 GA x 25 mm needle (Pneu- Dart Inc., Williamsport, PA, USA).
All personnel involved in the physical activities during the implementation of this project and who came into any kind of contact with the experimental animals were vaccinated against rabies with “Verorab” (Sanofi-Pasteur) according to standard pre-exposure vaccination protocols.

3.2.2.3 Transmission Study

Challenge virus

The virus used for the experimental transmission studies and to challenge the vaccinated animals was supplied by Dr. Thomas Müller and Dr. Conrad Freuling, Friedrich-Loeffler-Institut (FLI), Isle of Riems, Germany. It was originally derived from an isolate from brain tissue of a rabid kudu from Namibia, and supplied in two different doses, high ($10^{5.3}$ $MICLD_{50}$) and low dose ($10^{3.3}$ $MICLD_{50}$). The challenge virus was contained in 1 ml vials and transported by air to Namibia. Until further use, it was stored in liquid nitrogen at the CVL in Windhoek and transported to the holding facility on dry ice.

The challenge virus (Lab ID 23079) was initially isolated from the brain of a naturally infected kudu (240K09, GenBank accession JX473841) and genetically characterized (Scott et al., 2013). After three passages on mouse neuroblastoma cells (NA42/13), the inoculum had a titer of $10^{3.3}$ $MICLD_{50}$/ml and $10^{5.3}$ $TCID_{50}$/ml when assayed by intracerebral inoculation of mice and cell cultures, respectively. Verification of the genetic identity of the virus isolate after three serial passages in cell culture using next generation sequencing (Nolden et al., 2016) revealed an additional insertion of three nucleotides at positions 2475-2477 (AAC) in the intergenic region between the phosphoprotein and matrix protein gene. This specific sequence variation was confirmed by conventional Sanger sequencing of two independent PCRs.

The high dose challenge virus was administered to all vaccinated animals, control animals and animals used in the transmission study by i. m. injection of 0,5 ml bilaterally into the masseter muscles (Figure 15).
Initially 21 kudu were allocated for the transmission studies. They were held in 3 groups of five animals each plus one group of six animals. One animal had to be removed. Prior to infection, the animals had been adapted to captivity for a period of 88 days. Two animals in each group were infected with a high ($10^{5.3}$ TCID50) and a low ($10^{3.3}$ TCID50) dose of the kudu rabies virus isolate, respectively, while the remaining conspecifics served as contact animals. The virus was administered by intramuscular (i.m.) injection bilaterally in the masseter muscle at a volume of 0.5 ml per site. Following infection, the animals were observed at least twice daily for a period of 261 days for the occurrence of rabies-related symptoms and the development of such clinical signs was recorded. In case any symptoms, which clearly suggested rabies, such as bellowing, throwing head back, persistent swallowing movements, persistent licking, paresis and paralysis, hypersalivation, frothing at the mouth were observed, the animals were immediately sedated and humanely euthanized with an overdose of thiafentanil oxalate (10 mg/ml (Thianil, Wildlife Pharmaceuticals). Following euthanasia, brain samples were submitted to the CVL for analysis by FAT.

Figure 15: Administration of challenge virus into the masseter muscle of a kudu

3.2.2.4 Vaccination Studies

For oral vaccination, a vaccine strain with a very high safety profile was used. The vaccine strain SPBN GASGAS licensed for foxes and raccoon dogs (Freuling et al., 2017) is derived from SAD L16, a cDNA clone of the oral rabies virus vaccine strain SAD B19. SPBN GASGAS lacks the pseudogen (Ψ). Also, at two amino acid positions, 194 and 333, of the glycoprotein, all three nucleotides were changed; position 194 – AAT (Asn) → 170 TCC (Ser), position 333 – AGA (Arg) → GAG (Glu) (Faber et al., 2005). Furthermore, it contains an additional identical glycoprotein gene with modifications as described above. It has been postulated that the overexpression of the rabies virus glycoprotein not only increases the efficacy of vaccination but also the safety profile of the vaccine by reducing potential risk of reversion to virulence and enhancement of apoptosis (Faber et al., 2007, Faber et a., 2002). The vaccine virus was propagated on BHK21 BSR Cl13 cells and harvested after 48 hours. The cell-cultured vaccine material was subsequently ultrafiltrated 5 times and stabilized (140 ml antigen + 60 ml 177 GS8) to reach a final titre of $10^{8.1}$ FFU/ml.
The experimental oral vaccine was supplied by IDT Biologika GmbH, Dessau, Germany.

Twenty-five kudu were initially allocated for vaccination studies and held in 5 groups of four animals each and one group of five animals for easy handling and to prevent overcrowding of animals in pens (Table 5). Twenty animals completed the studies. The vaccination studies comprised of (i) direct oral administration (DOA) of the oral rabies virus vaccine construct SPBN GASGAS, and (ii) parenteral vaccination with a commercial inactivated rabies vaccine as control. Prior to vaccination, the animals were allowed a 24 days adaptation period in

captivity. In the DOA approach eleven animals (8 females and 3 males) in three groups received approx. 5.0 ml of the oral rabies virus vaccine construct SPBN GASGAS ($10^{8.1}$ FFU/ml). Only 10 animals completed this study, since one had to be euthanized due to a leg injury. In the control approach, 9 female animals divided over 3 groups received 2.0 ml of a commercially available inactivated rabies vaccine (Rabisin, Merial South Africa, Halfway House South Africa) by the i. m. route. All vaccinated animals were challenged 56 days post vaccination. (on the same day the animals were infected in the transmission study) using the high dose ($10^{5.3}$ TCID50) of the kudu rabies virus isolate described above by intra-muscular injection into the masseter muscle. Survival of vaccinated animals was monitored over a study period of 183 days post infection (p.i.). After the end of this period, surviving animals were immobilized for the final collection of blood samples. At the same time, they received an additional i.m. vaccination (Rabisin, Merial South Africa) and were subsequently released into a game-proof fenced game camp on Okosongoro.

Following the successful conclusion of the bait acceptance studies as described in chapter 3.2.2.5 a further study was conducted with the oral vaccine contained in pieces of bait. 11 animals used as naïve contact animals in a horizontal transmission experiment (Hassel et al., in press) were subsequently offered different vaccine baits containing a sachet from biodegradable foil (size: 4.6 x 1.6 x 0.7 cm) filled with 1.7 ml SPBN GASGAS vaccine virus ($10^{8.1}$ FFU/ml). The animals were divided into four groups and each group was offered a different bait type by piercing the baits on the thorns of the acacia shrubs within the outdoor section of the enclosure. Three experimental baits were used (see detailed description – bait studies); namely apple-flavoured corn meal and ground camel thorn tree pods mixed with gelatine. Of the latter, two different types were used; one with a very thin layer of bait matrix (dipped) and one with a thicker layer of bait matrix (poured). Finally, camel thorn tree pods were scooped out and a vaccine sachet was placed inside (Figure 18 upper right panel).

Sampling

Blood samples (Figure 16) were collected in plain vacuum serum tubes using 21 ga blood collection needles (BD Vacutainer, Becton Dickson, Fraga, Spain) by puncture of the jugular vein of immobilized kudu. Except for the animals delivered from the Namatanga Conservancy, they were taken on the day of capture to assess the immunological status of the animals. While in the transmission study additional blood samples were taken on day 261 p.i. from surviving animals, blood samples in the vaccination study were obtained on day 28 and 56 post vaccination (p.v.) as well as on day 184 p.i. from survivors of the challenge infection, using the same method and equipment described above for blood collection. Blood samples were also collected at the time of challenge. With each sampling 3 x 7 ml blood were collected from each animal. The serum was decanted into 2 ml cryovials and sent to the Friedrich-Loeffler-Institut, Germany, on dry ice. Duplicate serum samples of each animal of each sampling were retained and are currently stored at – 20º C as back-up samples.

Figure 16: Collecting blood samples from the jugular vein of immobilized kudu

Diagnostic Assays

Diagnostic testing was performed by CVL (FAT) and FLI (serology, FAT and PCR).

Rabies virus antigen in brain tissue of kudu was detected by FAT as described previously (Dean et al., 1996) using both polyclonal (OIE-RL Onderstepoort, Onderstepoort, South Africa) and commercial FITC-labelled monoclonal (SIFIN, Berlin, Germany) anti-rabies antibodies. Defined positive (PC, positive fox brain) and negative controls (NC, naïve cattle brain) were included in each test run. Inconclusive FAT results were confirmed by real-time polymerase chain reaction (rt-qPCR) (Hoffmann et al., 2010) and the rabies tissue culture infection test (RTCIT) (Webster et al., 1996), the latter with three consecutive passages to confirm a negative result.

Three different serological assays with modified cut-offs were used for the determination of rabies specific immune responses in animals. Virus neutralizing antibodies (VNA) were detected in a modified rapid fluorescence focus inhibition test (RFFIT) essentially as described by Moore et al., (2017) using the calibrated WHO international standard immunoglobulin (2nd human rabies immunoglobulin preparation, National Institute for Standards and Control, Potters Bar, UK) adjusted to 0.5 international units (IU). VNA titres were calculated using inverse interpolation and expressed in international units (IU/mL) (Müller et al., 2006). Presence of rabies specific binding antibodies were tested using a commercial blocking ELISA (BioPro Rabies ELISA, Czech Republic) following the manufacturer's instructions (Wasniewski et al., 2013).

As these serological assays were shown to have no unique identifiable cut-off (Moore et al., 2017), besides seropositive and seronegative results, an indeterminate result was introduced based on the results obtained for both assays as presented by Moore et al.

(2017). Hence, for the purpose of this study, VNA titres ranging between 0.25 and 0.75 IU/ml were considered indeterminate, while those <0.25 were considered negative and those >0.75 IU/ml positive. For the ELISA, an inhibition by the test serum between 34.72 – 44.14% compared to the negative controls was considered 'indeterminate', while an inhibition < 34.72% was considered negative and inhibition > 44.14 % positive, respectively. As a versatile and practical method for measuring the antibody level post vaccination, the Rapid Neutralizing Antibody (RAPINA) test (Nishizono et al., 2012) was also applied as a third method by following instructions of the manufacturer. The final scoring to decide if a serum sample was deemed antibody-negative or -positive to rabies virus was based on a weighted analysis of all results obtained by the 3 different assays. ELISA and RFFIT results were given more weight (++; --; ++/--) than the RAPINA test (+, -, +/-), for the latter is not considered a standard test for rabies serology yet; whereby ++ or + is seropositive, -- or - is seronegative and +/- or ++/-- indeterminate. Every + received a score of +1 and every – a score of -1. The total score was calculated by adding the values; a negative score (<0) was considered seronegative and a positive score (>0) as seropositive.

3.2.2.5 Bait Studies

For the initial stages of the bait acceptance studies, three experimental baits, none of which contained any vaccine, were prepared; Type 1 gelatin-based baits mixed with ground pods of the camel thorn tree, Type 2 gelatin-based baits containing pits of camel thorn tree pods and Type 3 apple-flavored corn meal baits. The size of these baits was 4.5 x 2.5 x 1 cm. As positive controls, pods of the umbrella thorn tree were used (Figure 17). During the first screening study, 8 pieces of a selected experimental bait were homogeneously mixed with 8 umbrella thorn tree pods (positive control) and placed in the food tray within the two selected pens holding each 4 female animals (two-food-preference test). After several hours bait removal was recorded and a new set of baits was placed.

Figure 17: Gelatin based bait (left) and acacia pods (right)

Distribution system

In a second screening study that was designed to include the bait distribution system, 24 baits were placed on the thorns of acacia bushes within the outside section of the holding pen overnight; 8 bait pieces each, of type 1, 2, and 3 plus 8 camel thorn tree pods (positive control) (Figure 18 upper left panel). The baits were distributed over several bushes in such a way that every bush contained at least 2 baits of each type. The next morning, bait disappearance was recorded. In addition, in two enclosures one observation video camera each and recording equipment (HIKVISION, China Electronics Technology Group, Hangzhou, China) were installed to monitor the animals.

In the third and last part of the placebo bait acceptance studies, the bait types 1 and 3 were placed in a similar way in several acacia trees close to a waterhole that was frequently visited by a large free-roaming male kudu antelope (Figure 18, lower panels). On three occasions, the experimental baits were pierced on the thorns in the early morning and bait uptake was monitored on two occasions by camera trap (Minox DTC 1000, Minox Wetzlar Germany) and personal observation on the third occasion.

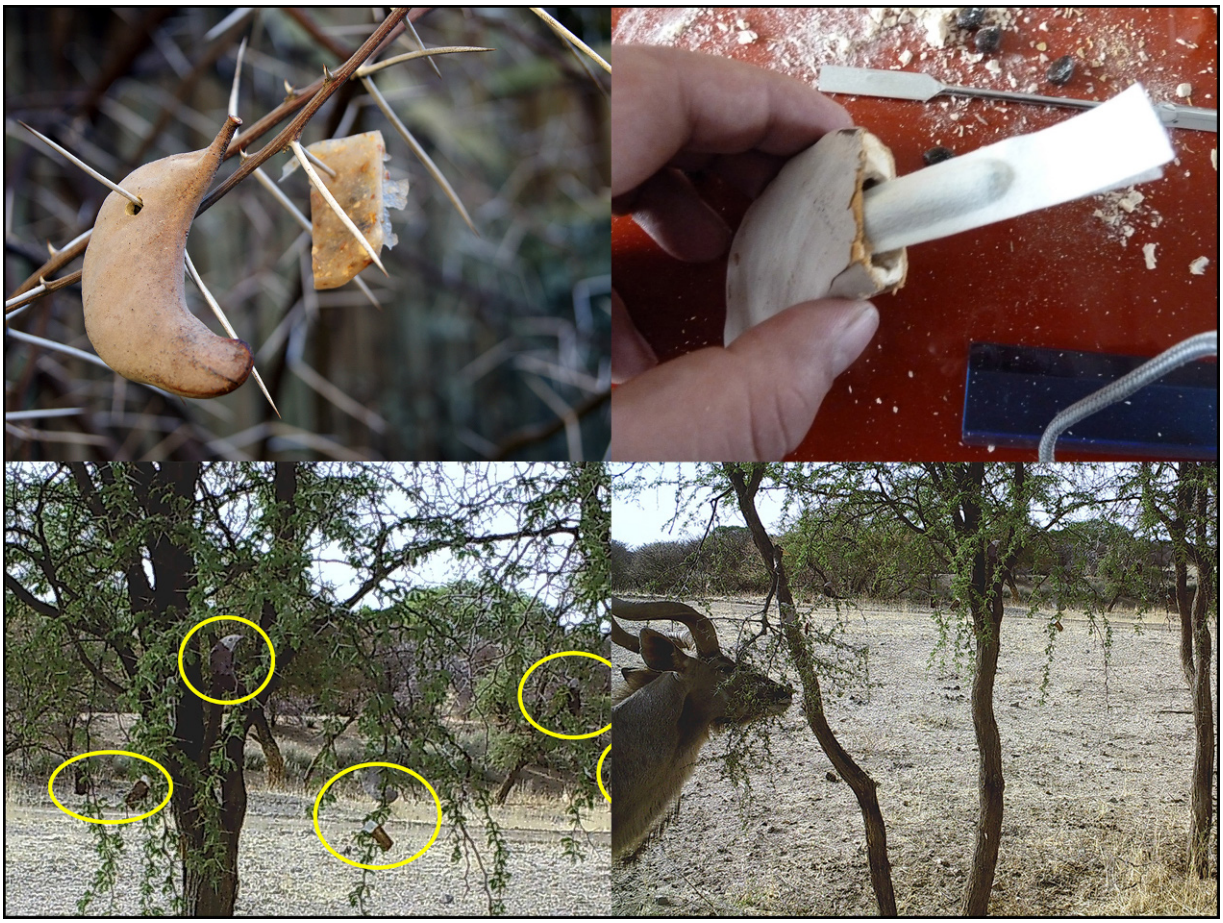

Figure 18: Vaccine baits, their presentation and uptake by kudu

clockwise from top left:

Camelthorn tree pod and gelatine- based bait inside boma
Vaccine blister inserted into hollowed out camelthorn tree pod (Picture: IDT Biologika)
Free-living kudu taking up bait
Gelatine-based bait pieces in thorn trees outside boma

The final component of the bait trials involved the distribution of vaccine laden pieces of bait to the experimental animals in the boma.

In this vaccine-bait study, 11 animals used as naïve contact animals in a horizontal transmission experiment (Hassel et al., in press) were offered different vaccine baits containing a sachet from biodegradable foil (size: 4.6 x 1.6 x 0.7 cm) filled with 1.7 ml SPBN GASGAS vaccine virus ($10^{8.1}$ FFU/ml). The animals were divided over 4 different groups and each group was offered a different bait type by piercing the baits on the thorns of the acacia shrubs within the outdoor section of the enclosure. Three experimental baits were used (see detailed description – bait studies); apple-flavoured corn meal and ground camel thorn tree pods mixed with gelatine. Of the latter, two different types were used; one with a very thin layer of bait matrix (dipped) and one with a thicker layer of bait matrix (poured). Finally, camel thorn tree pods were scooped out and a vaccine sachet was placed inside (Figure 18 upper right panel). The control animals for the challenge study (n=4) were the same animals used during the horizontal transmission experiment.

4. Results

4.1 Epidemiology of Kudu Rabies

4.1.1 Survey 1977-1980

Rabies cases

The estimated number of rabies cases was determined by results of routine examinations of specimens, case reports, questionnaires and observations made by field staff, including veterinarians, stock inspectors (animal health technicians), nature conservation specialists and farmers. The incidence of rabies in different species in areas where kudu contracted rabies was compared with the incidence in the same species in areas where rabies among kudu was not encountered (Barnard & Hassel,1981).

Table 6: Rabies cases in the part of Namibia where it occurred in kudu and in an adjacent area where kudu were not affected (Barnard & Hassel,1981)

Species	Rabies cases											
	Area with rabies in kudu						Area without rabies in kudu					
	1974	1975	1976	1977	1978	1979	1974	1975	1976	1977	1978	1979
Vectors												
Dog	2	1	4	3	4	5	1	0	0	0	0	2
Jackal	2	1	2	4	4	6	1	0	0	0	1	1
Other	0	0	0	0	4	5	0	0	0	3	0	4
Subtotal	4	2	6	7	12	16	2	0	0	3	1	7
Victims												
Cattle	4	0	10	5	18	20	7	3	4	1	6	10
kudu	0	0	0	2	36	58	0	0	0	0	0	0
Subtotal	4	0	10	7	53	78	7	3	4	1	6	10
Total	8	2	16	14	67	94	9	3	4	4	7	17

It was clear from the general trend of the disease in all species that there had been a gradual increase in rabies in jackal and dogs since 1976, while the increase in kudu was dramatic during the period 1978 – 1979 (Table 6).

Only 2 cases of rabies in kudu were confirmed during 1977, compared to 31 cases in domestic animals and 11 other wildlife species. During the next 2 years, 161 cases were confirmed in several species, of which 30 were vectors and 93 kudu originating from the Okahandja, Karibib, Omaruru and Windhoek districts. No other plains game species were involved during this period.

Spread of the disease

The first case confirmed in kudu (February 1977) originated from the farm “Bergquell” situated on the Swakop River (Figure 19). From this location, the disease spread in a westerly direction along the Swakop River to the Karibib district, where a case was confirmed on the farm “Uitdraai” in February 1978. After this east-west progression, the disease spread both northwards and southwards in the vicinity of the Swakop River. The first

case to be confirmed in the Omaruru district was on the farm “Kaliombo” in 1978 and in the Windhoek district in April 1979 in a specimen originating from the farm “Monte Christo”. It became evident that rabies in kudu occurred in contiguous areas and that the spread of the disease was progressive.

At first rabies in kudu did not spread eastwards beyond the North-South veterinary cordon fence (game proof fence for crawling and jumping game), but from November 1979 cases were confirmed on the east side of the VCF as well (Figure 19). By the end of 1979, cases were reported from the Otjiwarongo district. By 1980, it had reached the Outjo district in the North and Gobabis district in the East (Figure 19). Further spread northwards took place in 1981, when farms in the Grootfontein district were affected for the first time (Figure 19). In 1982, the disease occurred in the Tsumeb district, reaching the borders of the Etosha National Park.

Rabies cases were recorded in Etosha during 1983 and 1984 (Berry, 1993). There was also progression of the disease in the East in the Gobabis district (Figure 19). The infection spread in all directions except for the first 2 years, when it was contained by the central North – South VCF (Figure 19). By the middle of 1983, 90 cases of rabies in kudu had already been confirmed mainly in the North and the East (Figure 20, Table 19). 1983 is generally regarded as the peak of that epidemic.

Confirmed cases started to decline from 1984, reaching very low levels by 1986 (Figure 20, Table 19, Table 20, Table 21). The next epidemic of rabies in kudu started between 2001 and 2002 and has persisted ever since (Figure 20, Table 19, Table 20, Table 21).

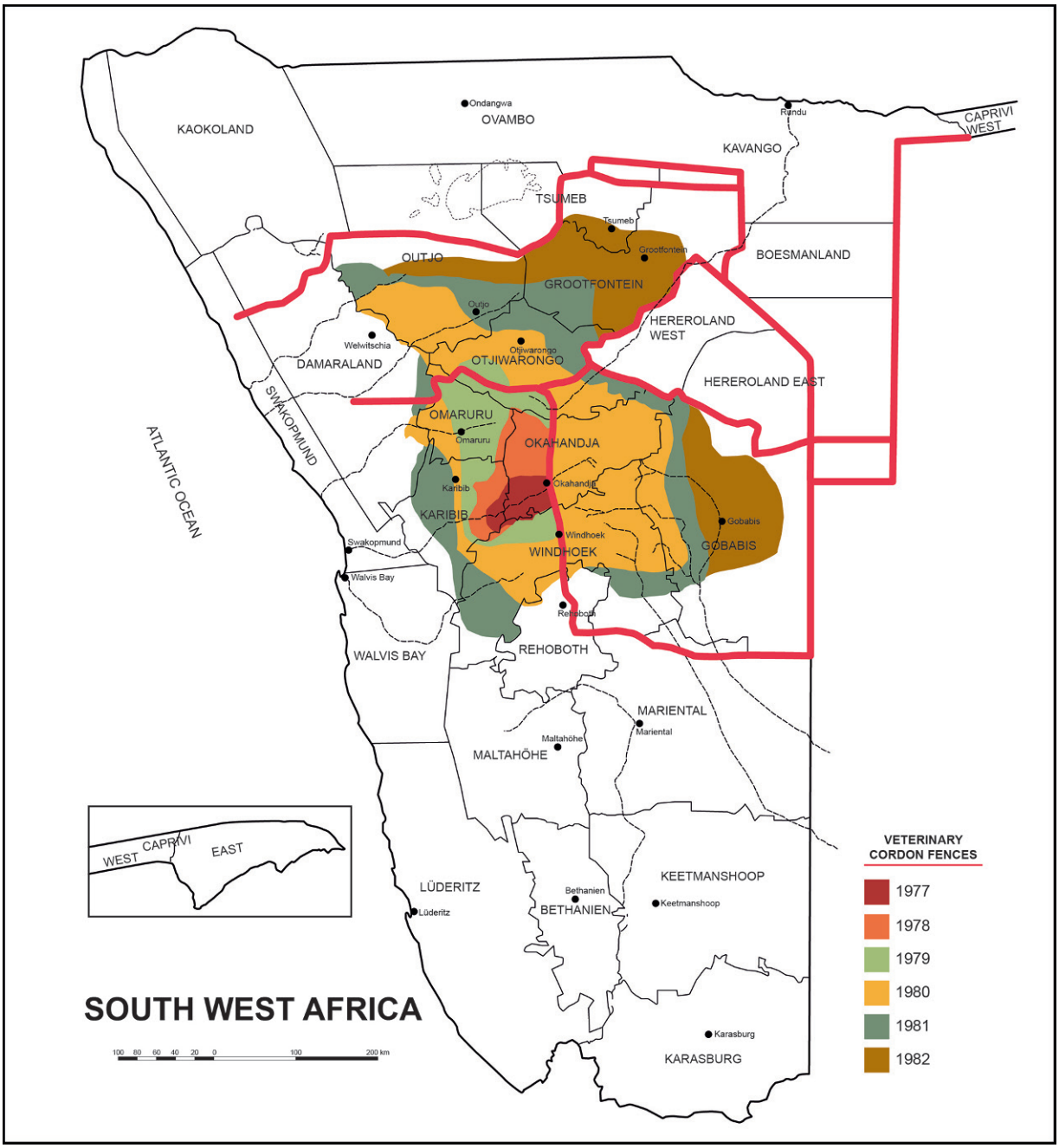

Figure 19: Map of Namibia (pre-independence) with districts, larger towns, veterinary cordon fences and the spatial progression of the first rabies epidemic in kudu between 1977 and 1982 (adapted from Barnard & Hassel, 1981 and Hassel, 1982)

Further results of the first survey

Completed questionnaires, covering the period 1977 – 1980, were received back from 143 farms, comprising 103 farming units (there were farmers owning more than one farm). This represented a 50% return rate of questionnaires. The most important findings can be summarized as follows:

1. The total surface area covered by the survey was 10 243,28 sqkm (1 024 328 hectares)

2. Estimated kudu population at the time of the start of the outbreak: 25 371
3. Density of kudu at the time: 1 kudu per 0.4 square kilometres (40 ha)
4. Number of carcasses counted by farmers: 4 735 or 15.7% of the total population
5. Number of sick kudu observed by owners: 189
6. Kudu population: An increase in the kudu population in the years prior to the outbreak was reported from 42 units (40.7%) while the remaining 61 units (59.3%) reported that the kudu population had remained static.
7. An increase in the jackal population was reported from 34 units (33.0 %).
8. 61 jackal either dead or with clinical rabies symptoms were reported on 20 units.
9. Sex distribution: 62.1% of units reported both sexes of kudu affected equally; 34.8% more male than female animals and 3.1% more female animals affected.

Clinical signs: The symptoms reported in the 189 cases as observed by farmers are summarized in Table 7. By far the most common symptoms were loss of fear or tame appearance (43.0%), various forms and degrees of paralysis (41.0%) and hypersalivation (37.1%). In nearly 10% of cases, the affected animals were aggressive. This is an important finding, emphasising that extreme caution needs to exercised, when dealing with clinical cases of rabid kudu, especially large male animals, which possess incredible body strength, and can therefore be extremely dangerous when aggressive.

Symptoms occurred alone or in combination or were progressive. 16 farmers reported finding one or more carcasses at or near water sources. Never were any kudu showing symptoms observed in the company of healthy kudu, nor in the company of other diseased kudu. This is different from later findings when more than one rabid kudu was found together.

Table 7: Clinical signs observed in 189 rabid kudu as reported in survey

Symptom	No. of affected animals	Percent
Loss of fear or tame appearance	81	43
Various forms of paralysis	78	41
Hypersalivation	70	37
Aggression	18	9.5
Abnormal vocalization	16	8.5
Inability to drink water	13	6.9
Poor condition	12	6.3
In the company with or aggressive towards domestic animals	8	4.2
Continuous tail wagging	8	4.2
Anxious facial expression	6	3.2
Biting or eating foreign objects	5	2.6
Walking into obstacles	4	2.1
Walking in circles	3	1.6
Ruffled or poor hair coat	2	1

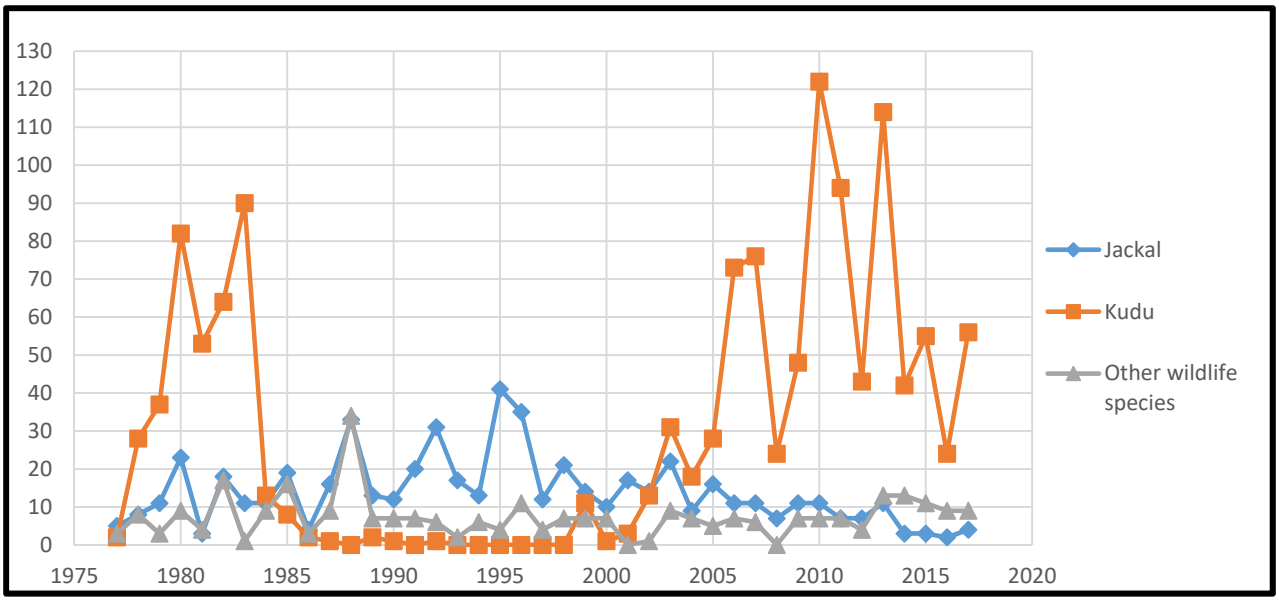

Figure 20: Confirmed rabies cases in wildlife, 1977 – 2017

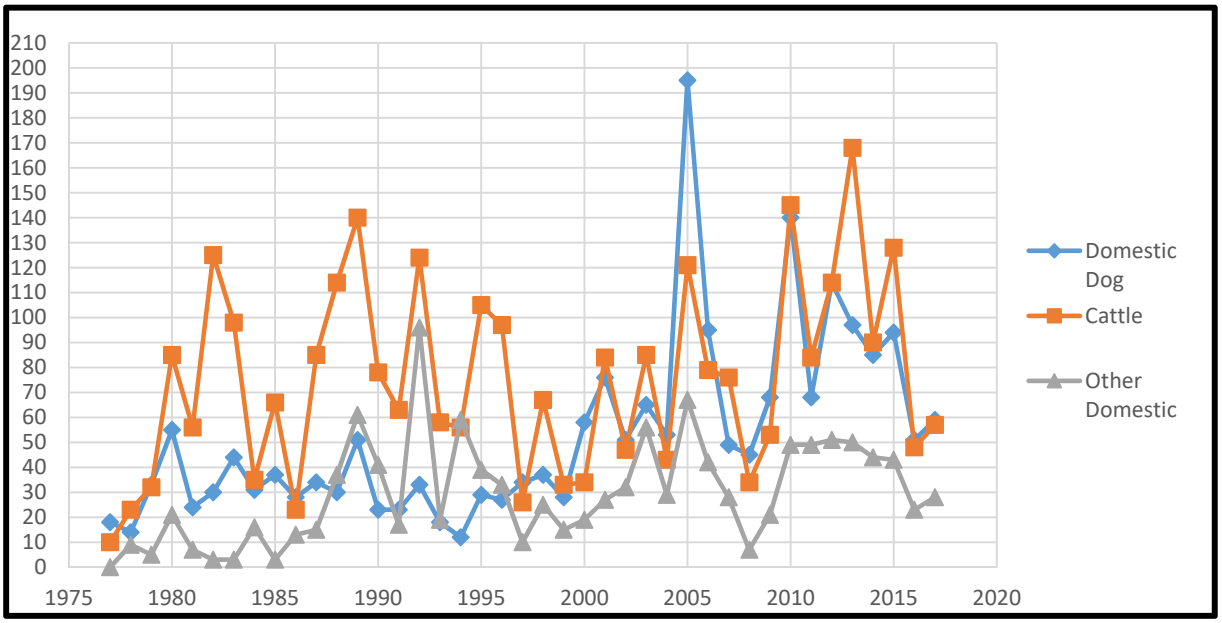

Figure 21: Confirmed rabies cases in domestic animals, 1977 – 2017

4.1.2 Survey 2009 - 2015

In the survey conducted for the period 2009 - 2015, 96 completed questionnaires were returned and evaluated. Of these, 83 reported rabies in kudu while in 13 instances the farmers indicated that no rabies in kudu occurred at the time. The total surface area covered in the second survey where rabies occurred in kudu was 5565.73 sqkm or 556 573 hectares of free hold commercial farmland. During the survey period, rabies in kudu occurred mainly on freehold farms in the central and northern areas of the country, with a few isolated foci in the southern parts, which marked the start of an epidemic in this part of the country for the first time (Figure 23). Losses on these farms varied from 10% to almost 100% of the kudu populations present on these farms at that time. Losses were particularly high on fully game-fenced units. It is also worthwhile to note that the spatial distribution of kudu rabies foci at that time covered virtually the complete area of the first epidemic, when the disease had taken six years of progressive spread to cover a similar surface area (Table 23, Table 24 in Addendum).

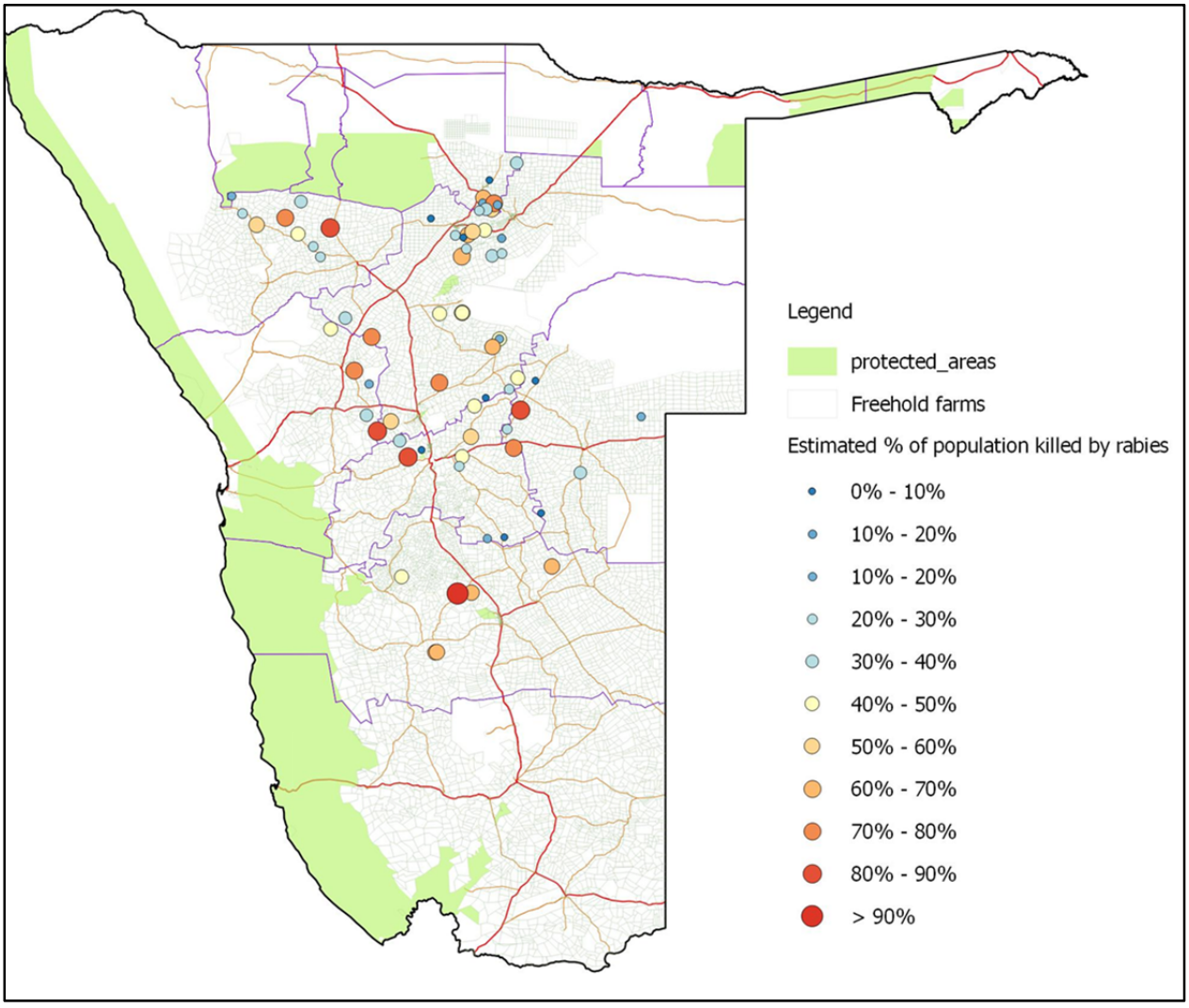

Figure 23: Map of Namibia with protected areas, freehold farms and estimated percentage of kudu populations succumbed to rabies, 2009 – 2015 (tables 23 and 24)

The majority of farmers who participated in this survey conducted regular game counts. This is required by law, in order to obtain a permit from the Ministry of Environment and Tourism, for a quota of game that can be hunted or captured and sold on a particular farm. Members of conservancies on freehold farms also agree to hold annual game counts to enable the implementation of an integrated game management plan for a particular conservancy.

During the time under review, the calculated losses amounted to 7750 kudu. 3388 kudu cadavers and 138 eland were counted by farmers, while they observed 669 clinically sick animals (Figure 24, Figure 27).

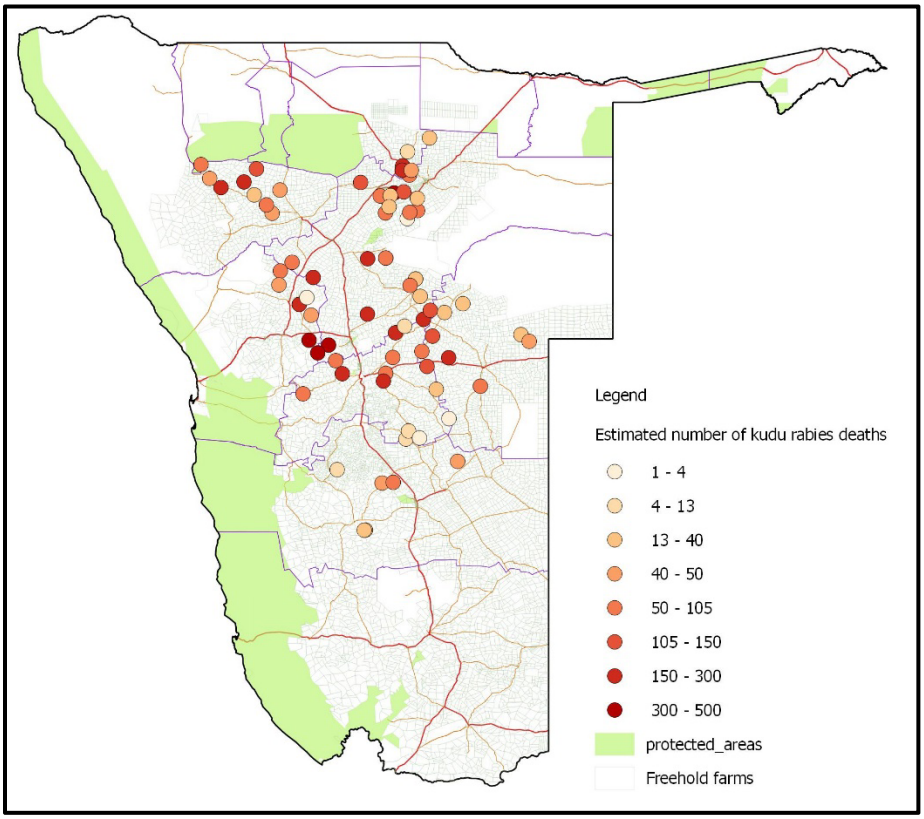

Figure 24: Map of Namibia with protected areas, freehold farms and estimated number of kudu fatalities, 2009 – 2015 (Tables 23 and 24)

Perceptions persist very widely among the farming community that only male kudu are affected by rabies. Both surveys reported in this thesis, however, paint a different picture and indicate that both sexes are affected, although the ratio differs on different farms (Figure 25) In most cases, both sexes were equally affected; 61% in the more recent survey (Figure 26) compared to 62.1% in the previous one. In a marked number of returned questionnaires, it was reported that more males than females were affected, with a smaller percentage reporting more affected females than males (Figure 26). The results of both studies prove conclusively that both sexes are affected equally in the majority of outbreaks.

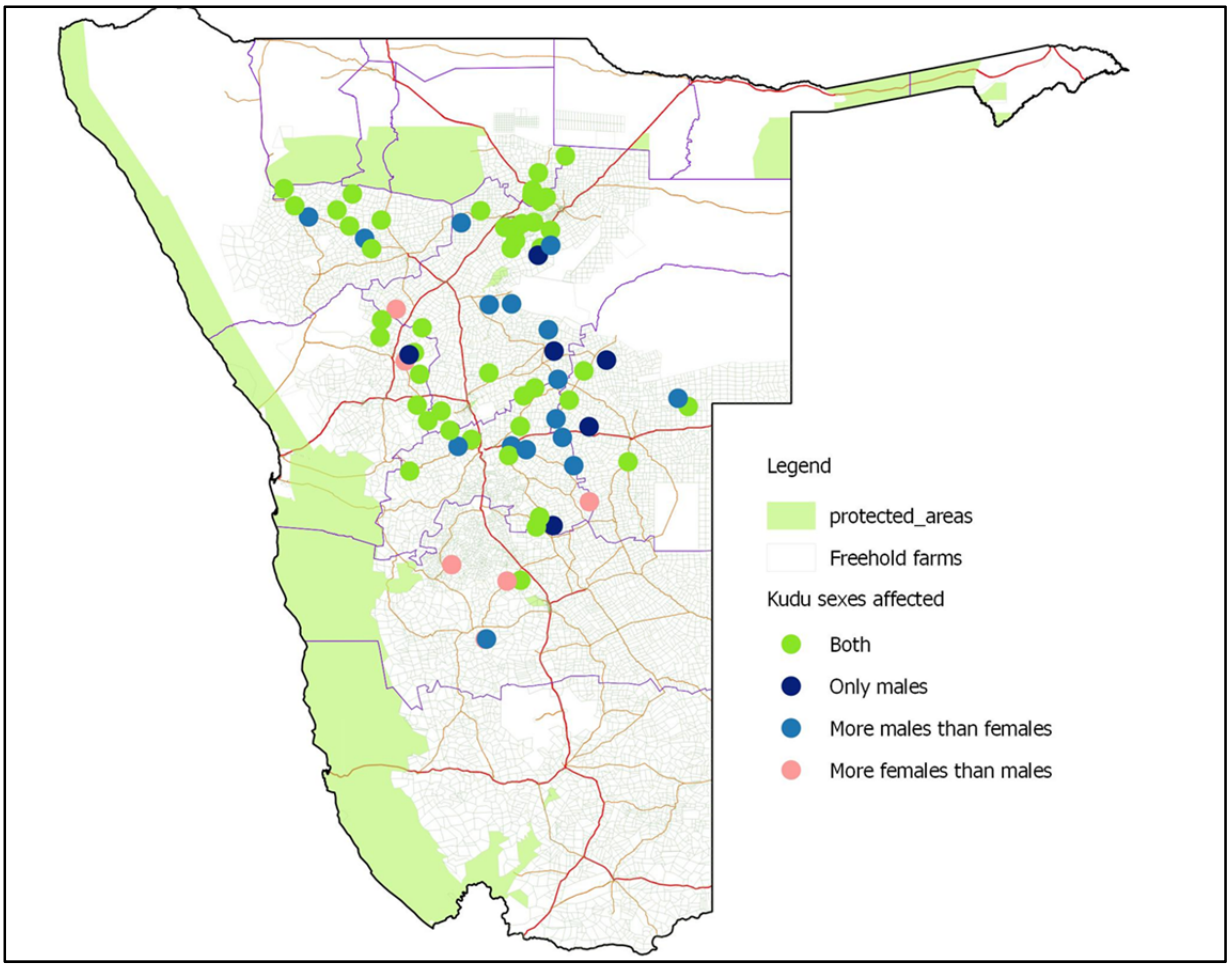

Figure 25: Map of Namibia with protected areas, free hold farms indicating the sex ratios of kudu affected by rabies 2009 – 2015 (Tables 23 and 24)

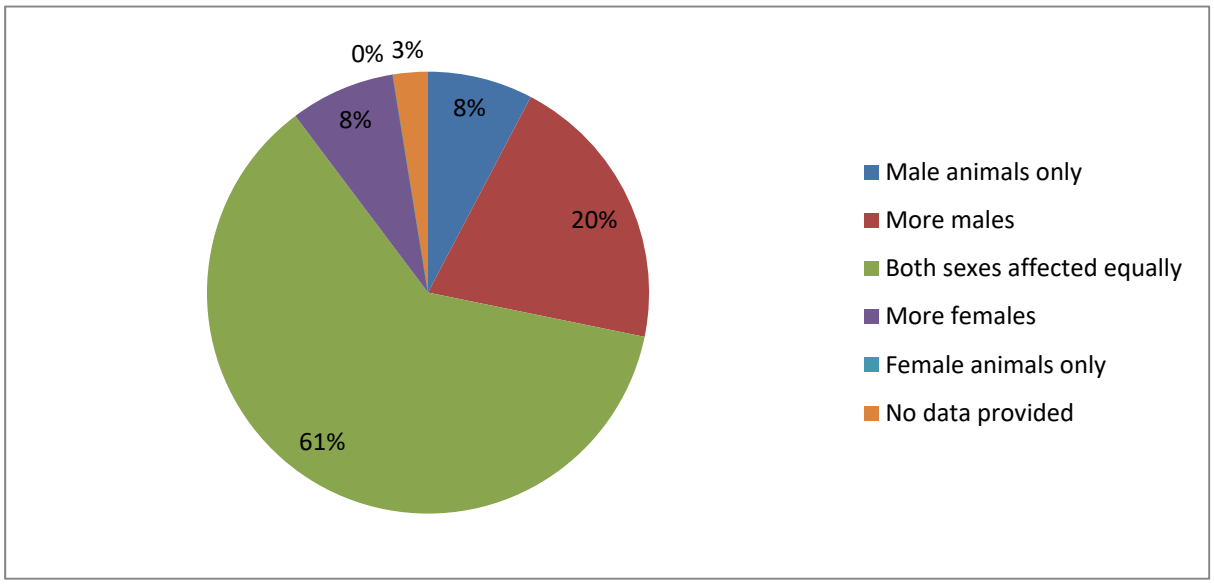

Figure 26: Sex ratio of kudu on freehold farms affected by rabies, 2009 - 2015

78 returned questionnaires reported on the sex ratio of affected animals as follows: in 6 instances only male animals were affected, more male than female animals in 16 cases, both sexes affected equally, 48 cases. No farmer reported that only females were affected, while two did not comment on the sex ratio.

In 34 cases, farmers reported that other animal species were also affected by rabies on the same farms where rabies had occurred in kudu. (Figure 27) The majority involved eland antelope (*Taurotragus oryx*) (n= 21; 62%), while on a number of farms cattle (n=10; 29%), one unnamed wild herbivore (3%) and two wild carnivores (6%) were affected (Figure 27). This trend persists during the current outbreak, with cases in kudu, eland and cattle found rabies-infected on single farms.

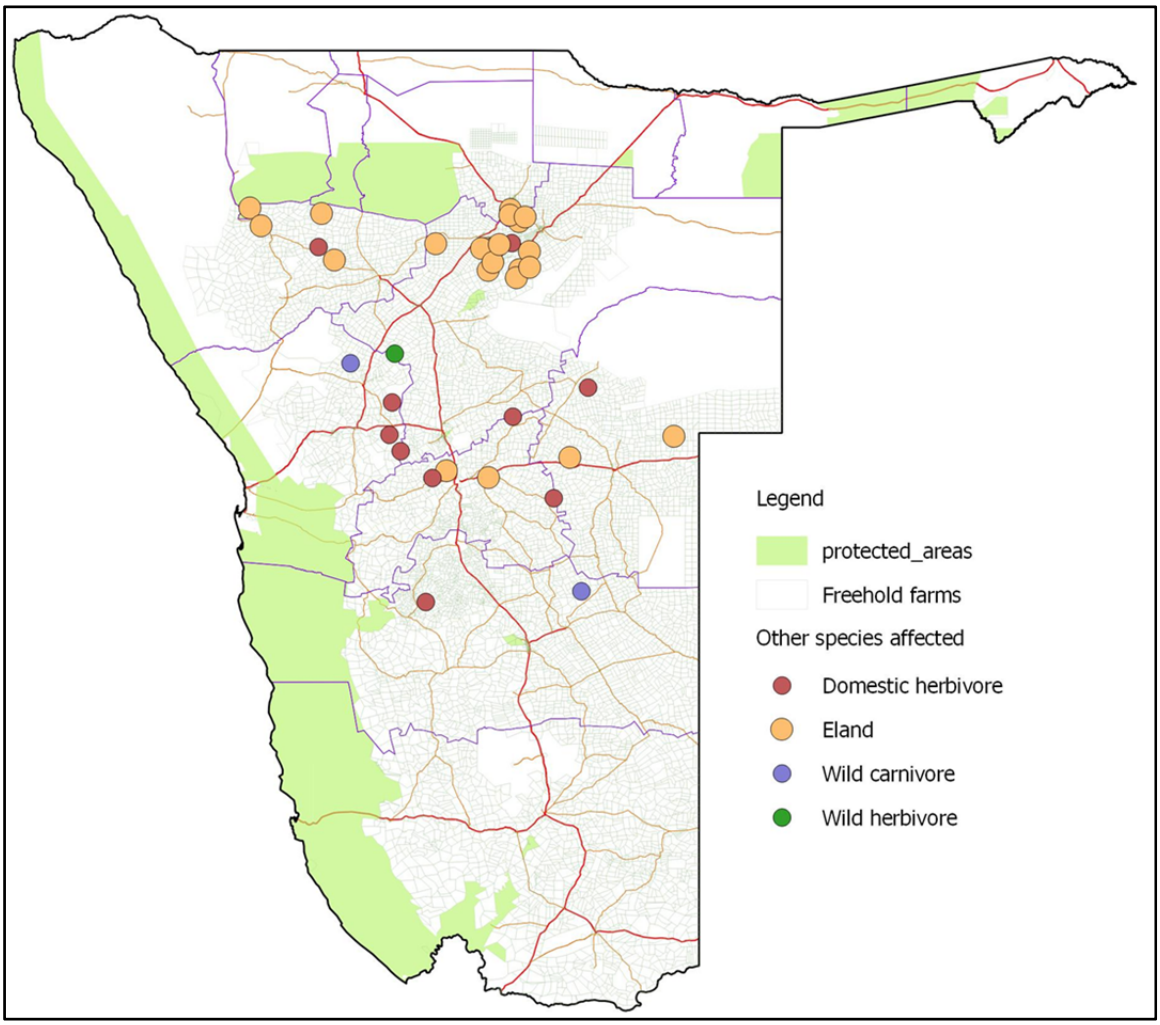

Figure 27: Map of Namibia with protected areas, freehold farms and other species affected on farms where rabies occurred in kudu, 2009 – 2015 (Tables 23 and 24)

In this survey we were unable to show a correlation between population densities and kudu mortalities nor a correlation between game proof fences and mortalities.

Rabies data

In addition to the two surveys and as part of the epidemiological investigation, all available data contained in the records of the CVL and in the reports of the Epidemiology Section of DVS were compiled into tables of confirmed rabies cases for a continuous period of 1977 up to and including 2017 (Figures 20, 21). (Table 19, Table 20, Table 21 in Addendum).

Figure 20 represents wildlife rabies and compares the annual number of confirmed cases in kudu, jackal and all other wildlife, respectively. This was done to demonstrate possible links between peaks of rabies in the traditional wildlife reservoir and vector, the black-backed jackal and peaks of the disease in spill-over populations.

Figure 21 represents dog rabies and compares the annual number of confirmed cases in dogs, cattle and other domestic animals. Once again, the aim is to demonstrate a possible link between rabies in dogs and "spill-over" populations.

Furthermore, CVL provided data regarding the number of kudu samples which tested negative for FAT between 2011 and 2016. These data show that each year a number of samples from suspected cases tested negative for rabies. These numbers varied from 14% from 54.7% of the total number of samples tested.

4.2 Animal Studies

4.2.1 Experimental Studies

Transmission experiments (non-bite)

Virus titration: The period between the collection of specimens and virus titration varied from 4 – 16 days (Table 8). The titres in the brain sample of kudu varied from 10^4 -10^7 mouse LD 50/ml. In 3 out of 3 titrated cases, the titres of virus were $10^{0,7}$ – $10^{2,9}$ mouse LD 50/ml higher than those in the salivary glands. The virus titres in the blood of case 570/80 was 10 mouse LD50/ml (Barnard et al., 1982).

Table 8: Rabies virus titres in kudu that died of natural rabies (Barnard et al., 1982)

Case No.	Time* (days)	Brain	Salivary glands	Saliva	Blood
1253/79	4	6.5**	N***	N	N
77/80	6	7.0	N	N	N
94/80	9	4.8	N	N	N
100/80	10	4.8	N	N	N
448/80	16	4.0	4,4	N	N
350/80	16	4.1	3.5	N	N
570/80	4	6.1	3.5	6.4	1.0
660/80	5	N	3.8	4.5	N
662/80	5	N	2.8	4.0	N

*: Time from collection to titration

**: Expressed as log 10 mouse LD50/ml

***: N = not available for determination

Infectivity of kudu saliva: Two out 4 kudu (Table 9), infected experimentally by instillation of infected saliva into the buccal and nasal cavities, died of rabies 21 or 33 days after exposure, respectively. Rabies virus was isolated from their brains and salivary glands. Both cattle infected with saliva survived for more than 15 months (Barnard et al., 1982).

Table 9: Reaction of kudu and cattle to experimental exposure to RABV infected saliva (Barnard et al., 1982)

Animal	Outcome	FAT	Virus Isolation
kudu 1	Survived	•	
kudu 2	Survived	•	Saliva negative day 33
kudu 3	Died day 33	positive	Brain & salivary gland
kudu 4	Died day 21	positive	Brain & salivary gland
cattle 1	Survived	•	
cattle 2	Survived	•	

Infectivity of HEP Flury virus: Only kudu reacted after exposure to the HEP Flury virus of tissue culture origin by production of virus-neutralizing antibodies in 4 out of 6 kudu exposed (Table 10) (Barnard et al., 1982).

Table 10: Serological response of kudu and cattle following oral and nasal exposure to HEP Flury virus (Barnard et al., 1982).

Animal	Route of exposure	Virus-neutralizing antibody titre on		
		Day 30	Day 60	Day 90
kudu 1	2 x 10^6 MLD 50 Mucosal surface	< 1:4	1:4	< 1:4
kudu 2		< 1:4	1:12	1:12
kudu 3		1: 12	1:4	1:4
cattle 1		< 1:4	< 1:4	< 1:4
cattle 2		< 1:4	< 1:4	< 1:4
cattle 3		< 1:4	< 1:4	< 1:4
kudu 4	3 x 10^6 MLD 50 Drinking water	< 1:4	1:6	< 1:4
kudu 5		< 1:4	< 1:4	< 1:4
kudu 6		< 1:4	< 1:4	< 1:4
cattle 4		< 1:4	< 1:4	< 1:4
cattle 5		< 1:4	< 1:4	< 1:4
cattle 6		< 1:4	< 1:4	< 1:4

Cattle 4, 5 and 6 were re-exposed after 30 days.

Susceptibility of mice: None of the mice fed on infected kudu brain tissue developed signs of rabies during an observation period of 28 days (Table 11) (Barnard et al., 1982).

Table 11: Susceptibility of mice to oral administration of RABV infected kudu brain

Virus isolate	Virus titre	Deaths/exposed mice
1253/79	6.5 *	0/12
1016/79	4.0	0/12
1106/79	5.5	0/12
79/80	7.0	0/36
94/80	4.8	0/16

* Expressed as log 10 mouse LD 50/ml

4.2.2 Vaccination Studies

Five of 25 animals from the vaccination groups were considered antibody positive prior to vaccination (day of capture; Table 14). All 9 animals that were vaccinated by the parenteral route (i.m.) developed a strong immune response as measured by the three separate assays and survived the challenge infection (Table 12). With 92.1, 93.6 and 94.1 %, the ELISA mean percent blocking values, the parenterally vaccinated kudus showed almost complete blocking at all three time points p.v. Geometric mean titres (GMT) of VNAs were 0.32, 4.18, 9.90 and 3.38 IU/ml on day 0, 28, 56 p.v. and 183 p.i., respectively.

By contrast, only 3 of 10 kudu vaccinated by DOA survived the challenge, although none of these 3 animals had detectable levels of antibodies prior to challenge (Table 12). ELISA mean percent blocking values in animal sera of the DOA group were below the cut-off (40%) on day 0 (23.8) and prior to challenge (30.3, 31.4). The GMTs in the VNA of orally vaccinated animals were 0.21, 0.37, 0.45, and 1.27 IU/ml at day 0, 28, 56 p.v. and 183 p.i., respectively (Table 15). Two animals developed an immune response p.v., but succumbed to infection, while 3 animals with no measurable antibody response prior to challenge survived the challenge and had detectable rabies-specific antibodies on day 183 p.i. as measured in at least two assays. Of the 7 orally vaccinated animals that succumbed to rabies between 12 and 26 days post infection (p.i.) (Figure 28), 6 were FAT-positive, while one FAT-negative animal tested positive in rt-PCR.

All parenterally vaccinated animals and the three survivors of the orally vaccinated group were re-vaccinated parenterally, double ear-tagged and re-homed 187 days post challenge on the spot.

Table 12: Group composition and survival after infection/challenge.

	Number of animals									
Study	Group	Total	Removed	Used	Female	Male	Day	Challenge dose $TCID_{50}$/mL	Survival	%age
Transmission	High dose	4	-	4	4	-	0	$10^{5.3}$	0/4	0
	Low dose	4	-	4	4	-	0	$10^{3.3}$	1/4	25
	contact	13	1	12	12	-	0	-	11/12	92
Vaccination	parenteral	12	3	9	9	-	56	$10^{5.3}$	9/9	100
	DOA	13	3	10	7	3	56	$10^{5.3}$	3/10	30

4.2.3 Transmission Studies

Two infected and two contact animals were rated antibody-positive prior to infection (day of capture; Table 13). All four animals inoculated with high dose ($10^{5.3}$ TCID50) of the kudu RABV isolate succumbed to infection. The animals died on days 12, 13, 15 and 16 p.i. One animal inoculated with the low dose ($10^{3.3}$ TCID50) survived the 261 days observation period. The remaining three died or had to be euthanized 93, 233 and 245 days p.i. (Figure 28) Hypersalivation, a typical symptom of natural rabies infection in kudu, was not observed, only mild frothing at the mouth in two cases. Six animals that had succumbed to the infection were FAT positive, while two were regarded inconclusive in that assay, but positive in RT-qPCR. All but two of the contact animals survived the 261 days observation period. Two of 12 contact animals died 99 and 156 days p.i., however, only in the latter infection, viral antigen could be detected by FAT. Sequencing of the RABV isolate of the FAT-positive contact animal revealed a 100% sequence identity with the challenge virus including the insertion.

Table 13. Individual immune response of infected and contact animals from the transmission study

		Day of capture				Day 261 p.i.			
Animal	Status	ELISA	RAPINA	RFFIT	Score	ELISA	RAPINA	RFFIT	Score
1-1	Contact	--	-	--	neg	--	-	++ --	neg
1-2	Control, low dose	--	-	--	neg				
1-3	Control, high dose	--	-	--	neg				
1-4	contact	++ --	+	--	neg	--	+	++	pos
1-5	contact	--	+	++ --	neg				
1-6	**removed**	--	-	--	neg				
1-7	contact	--	-	++ --	neg	--	-	++	neg
1-8	contact	--	-	++ --	neg	--	+	++	pos
1-9	contact	--	-	--	neg	--	-	--	neg
1-10	contact	--	-	--	neg		+	++	pos
1-11	contact	--	-	--	neg				
1-12	Control, high dose	++ --	-	++ --	neg				
1-13	Control, high dose	--	-	++ --	neg	--			
1-14	contact	--	-	--	neg		-	++ --	neg
1-15	Control, low dose	++	-	++ --	pos				
1-16	Control, low dose	++		++ --	pos				
1-17	contact	--	+	++ --	neg	++	+	++	pos
1-18	Control high dose	++	-	++ --	neg				
1-19	contact	++	+	--	pos	++	+	++ --	pos
1-20	contact	--	+	--	pos	++	+	++	pos
1-21	Control, low dose	++ --	-	--	neg	++	+	++	pos

Table 14. Immune response of animals from the vaccination study immunized DOA and i.m. respectively

		day of capture				day 28 p.v.				day 56 p.v.				day 183 p.i.			
route	Animal ID	ELISA	RAPINA	RFFIT	score	ELISA	RAPINA	RFFIT	score	ELISA	RAPINA	RFFIT	score	ELISA	RAPINA	RFFIT	score
i.m.	2-1	++	+	++ - -	pos	++	+	++	pos	++	+	++	pos	++	+	++	pos
	2-2	- -	+	++ - -	neg	++	+	++	pos	++	+	++	pos	++	+	++	pos
	2-3	- -	-	- -	neg	++	+	++	pos	++	+	++	pos	++	+	++	pos
	2-4	- -	-	- -	neg	++	+	++	pos	++	+	++	pos	++	+	++	pos
	2-5	++	-	++ - -	pos	###											
	2-6	- -	-	- -	neg	###											
	2-7	++	-	- -	neg	++	+	++	pos	++	+	++	pos	++	+	++	pos
	2-8	++ - -	+	++	pos	++	+	++	pos	++	+	++	pos	++	+	++	pos
	2-9	++ - -	-	++ - -	neg	++	+	++	pos	++	+	++	pos	++	+	++	pos
	2-10	++ - -	-	- -	neg	###											
	2-11	- -	+	- -	neg	++	+	++	pos	++	+	++	pos	++	+	++	pos
	2-12	- -	+	++	pos	++	+	++	pos	++	+	++	pos	++	+	++	pos
DOA	3-1	- -	-	- -	neg	++ - -	-	- -	neg	-	-	++ - -	neg				
	3-2	- -	+	- -	neg	++	+	++ - -	pos	++	+	++	pos				
	3-3	++ - -	+	- -	neg	++	+	++	pos	++	+	++ - -	pos				
	3-4	- -	-	- -	neg	++ - -	-	++ - -	neg	- -	-	- -	neg	###			
	3-5	- -	-	++ - -	neg	- -	-	++ - -	neg	- -	-	++ - -	neg				
	3-6	++ - -	+	++ - -	pos	++	-	- -	neg	++ - -	-	- -	neg	++	+	++	pos
	3-7	- -	-	- -	neg	- -	-	- -	neg	- -	-	++ - -	neg				
	3-8	- -	-	- -	neg	- -	-	++ - -	neg	- -	-	- -	neg	++	+	++	pos
	3-9	- -	+	- -	neg	- -	-	++ - -	neg	- -	-	++ - -	neg				
	3-10	- -	-	++ - -	neg	- -	-	++ - -	neg	- -	-	++ - -	neg				
	3-11	- -	-	- -	neg	- -	-	++ - -	neg	- -	-	++ - -	neg	++	-	++	pos
	3-12	- -	-	- -	neg	###											
	4-3	- -	-	- -	neg	###											

Legend: ### = animals were removed

Table 15: Summary Clinical records of experimental animals

Animal No	Conservancy Of Origin	Date of arrival at the boma	Group/ Purpose	Oral vaccination	I.M. vacc	High Dose Challenge post vacc	Exp. Infect.	Fate	FATR	PCR
1-01	Namatanga	18/07/15	Control/Exper. Transm.	Bait 24/06/16	-	-	-	Vaccinate & Release 26/08/16	-	-
1-02	Namatanga	18/07/15	Control/Exper. Transm.	-	-	-	**HD 06/10/15**	Died 19/10/15	**pos**	
1-03	Namatanga	18/07/15	Control/Exper. Transm.	-	-	-	**LD -06/10/15**	Died 09/06/16	**pos**	
1-04	Namatanga	18/07/15	Control/Exper. Transm.	Bait 24/06/16	-	-	-	Vaccinate & Release 26/08/16	-	-
1-05	Namatanga	18/07/15	Control/Exper. Transm.	-	-	-	-	Died 10/03/16	neg	
1-06	Namatanga	18/07/15	Control/Exper. Transm.	Calf; Collapsed after capture. Died on arrival; did not enter trial						
1-07	Namatanga	18/07/15	Control/Exper. Transm	Bait 24/06/16	-	-	-	Vaccinate & Release 26/08/16	-	-
1-08	Namatanga	18/07/15	Control/Exper. Transm	Bait 24/06/16	-	-	-	Vaccinate & Release 26/08/16	-	-
1-09	Namatanga	18/07/15	Control/Exper. Transm	Bait 24/06/16	-	-	-	Vaccinate & Release 26/08/16	-	-
1-10	Namatanga	18/07/15	Control/Exper. Transm	Bait 24/06/16	-	-	-	Vaccinate & Release 26/08/16	-	-
1-11	Namatanga	18/07/15	Control/Exper. Transm	14/01/16. Haemorrhagic enteritis; euthanasia; coccidiosis					neg	-
1-12	Namatanga	18/07/15	Control/Exper. Transm	-	-	-	**HD 06/10/15**	Euth. 18/10/15	**pos**	-
1-13	Namatanga	18/07/15	Control/Exper. Transm	-	-	-	**HD 06/10/15**	Euth. 22/10/15	neg	**pos**

1-14	Namatanga	18/07/15	Control/Exper. Transm	Bait 24/06/16	-	-	-	Vaccinate & Release 26/08/16	-	-
1-15	Namatanga	18/07/15	Control/Exper. Transm	-	-	-	**LD 06/10/15**	Died rabies 01/16	Sample decomp.	
1-16	Namatanga	18/07/15	Control/Exper. Transm	-	-	-	**LD 06/10/15**	Died rabies 27/05/16	**pos**	-
1-17	Namatanga	18/07/15	Control/Exper. Transm	Bait 24/06/16	-	-	-	26/08/16	-	-
1-18	Richtberg	20/07/15	Control/Exper. Transm	-	-	-	**HD 06/10/15**	Died rabies 21/10/15	**pos**	**pos**
1-19	Richtberg	20/07/15	Control/Exper. Transm	Bait 24/06/16	-	-	-	Vaccinate & Release 26/08/16	-	-
1-20	Richtberg	20/07/15	Control/Exper. Transm	Bait 24/06/16	-	-	-	Vaccinate & Release 26/08/16	-	-
1-21	Richtberg	20/07/15	Control/Exper. Transm	Bait 24/06/16	19/08/16	-	**LD 06/10/15**	Vaccinate & Release 26/08/16	-	-
2-01	Namatanga	19/07/15	I.M. vacc	-	11/08/15	06/10/15	-	Vaccinate & Release	-	-
2-02	Namatanga	19/07/15	I.M. vacc	-	11/08/15	06/10/15	-	Vaccinate & Release	-	-
2-03	Namatanga	19/07/15	I.M. vacc	-	11/08/15	06/10/15	-	Vaccinate & Release	-	-
2-04	Namatanga	19/07/15	I.M. vacc	-	11/08/15	06/10/15	-	Vaccinate & Release	-	-
2-05	Namatanga	19/07/15	20/07/15; Died; Capture Stress; did not enter trials							
2-06	Namatanga	19/07/15	20/07/15; Died; Capture Stress; did not enter trials							
2-07	Namatanga	19/07/15	I.M. vacc	-	11/08/15	06/10/15	-	Vaccinate & Release	-	-
2-08	Namatanga	19/07/15	I.M. vacc	-	11/08/15	06/10/15	-	Vaccinate & Release	-	-
2-09	Namatanga	19/07/15	I.M. vacc	-	11/08/15	06/10/15	-	Vaccinate & Release	-	-
2-10	Namatanga	19/07/15	20/07/15; died broken neck; did not enter trials							
2-11 Ex 4-01	Richtberg	20/07/15	I.M. vacc	-	11/08/15	06/10/15	-	Vaccinate & Release	-	-
2-12 Ex 4-02	Richtberg	20/07/15	I.M. vacc	-	11/08/15	06/10/15	-	Vaccinate & Release	-	-

3-01	Kalkfeld	20/06/15	Oral Vacc DOA	09/0715	-	06/10/15	-	Died Rabies 18/10/15	**pos**	-
3-02	Kalkfeld	20/06/15	Oral Vacc DOA	09/0715	-	06/10/15	-	Died Rabies 01/11/15	**neg**	**pos**
3-03	Kalkfeld	20/06/15	Oral Vacc DOA	09/0715	-	06/10/15	-	Euth Rabies 21/10/15	**neg**	**neg**
3-04	Kalkfeld	20/06/15	Oral Vacc DOA	09/0715	Right front limb injury; paralysis; euthanasia 10/09/15; did not continue trial					
3-05	Kalkfeld	20/06/15	Oral Vacc DOA	09/0715	-	06/10/15	-	Euth Rabies 22/10/15	**neg**	
3-06	Kalkfeld	20/06/15	Oral Vacc DOA	09/0715	-	06/10/15	-	Survived Vaccinate & Release 26/08/16	**-**	**-**
3-07	Kalkfeld	20/06/15	Oral Vacc DOA	09/0715	-	06/10/15	-	Died rabies 19/10/15	**pos**	-
3-08	Kalkfeld	20/06/15	Oral Vacc	09/0715	-	06/10/15	-	Survived Vaccinate & Release 26/08/16	-	-
3-09	Namatanga	18/07/15	Oral Vacc DOA	11/08/15	-	06/10/15	-	Died rabies 20/10/15	**pos**	
3-10	Namatanga	18/07/15	Oral Vacc DOA	11/08/15	-	06/10/15	-	Died rabies 24/10/15	**pos**	
3-11	Namatanga	18/07/15	Oral Vacc DOA	11/08/15	-	06/10/15	-	Survived Vaccinate & Release 26/08/16	-	-
3-12	Richtberg	20/07/15	Oral Vacc DOA	01/08/15 Euthanasia; broken jaw; did not enter trial						
4-03	Richtberg	20/07/15	21/07/15 died capture stress/ capture myopathy; did not enter trial							

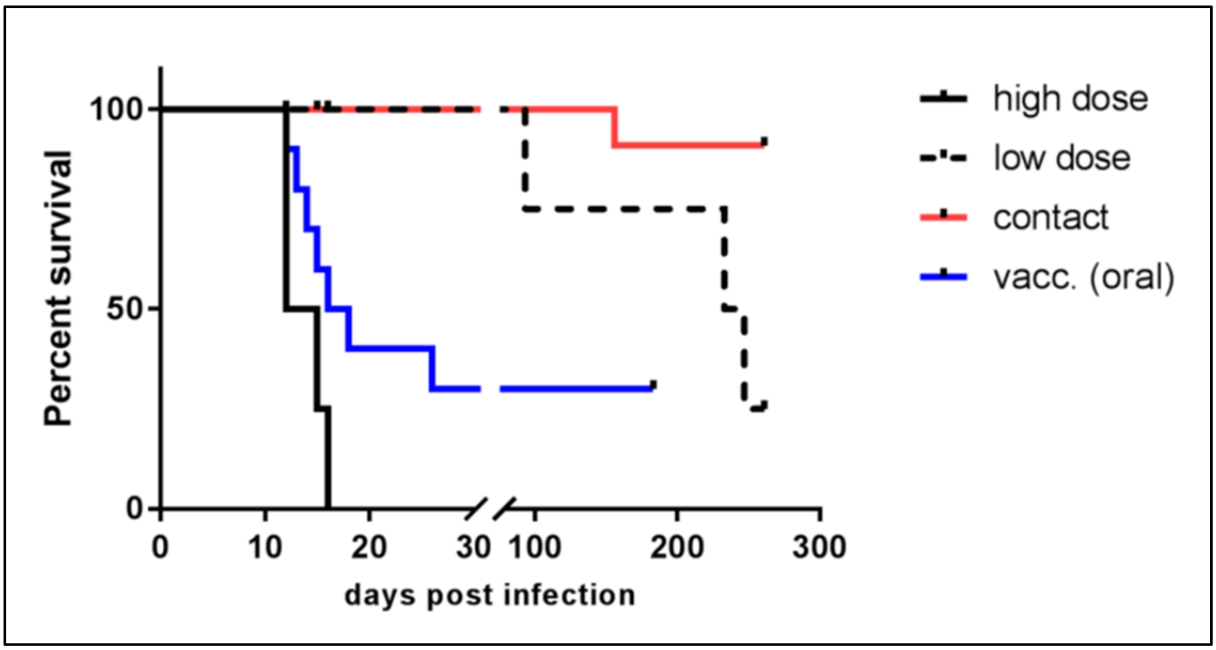

Figure 28: Suvival of different groups of experimental animals following infection and challenge with RABV as well as in contact.

The 9 animals of the parenteral vaccination group (control) are not included in the above figure since they all survived.

4.2.4 Bait Trials

The results of the pilot two-food-preference test are summarized in Table 16. During the second screening study with the baits, the animals had to locate and take the baits themselves from the acacia bushes. At this stage, the pens were not yet equipped with cameras, so bait removal could only be assessed by indirect observations. The next morning, all 24 baits in pen 1 were gone and no bait fragments were found of the floor. In pen 2, with the exception of three baits on the floor, one of each type, also here all baits had disappeared.

Table 16: Results of the first two-food-preference test

Time period	Bait type	Pen 1 (n/N) *		Pen 2 (n/N) *	
		bait	pos. control	bait	pos. control
09:40 – 11:30	Pits	0/8	3/8	0/8	0/8
11:45 – 14:30	Pods	8/8	1/8	8/8	1/8
14:45 – 09:00	Corn Meal	8/8	8/8	8/8	8/8

The umbrella thorn tree pods were used as positive controls

*- n – baits removed; N – baits placed

Results of trials with bait containing vaccine

The removal of the vaccine-loaded baits pierced on the thorns of the shrubs is summarized in Table 17. Bait acceptance in all groups, except for group C, was good. 90% and 85% of the bait had been removed by groups A and D respectively within 3 hours of placement, while 100% was removed by group A and 85% each by groups B and D after 24 hours. All baits offered to the animals in group C were still present after 24 hours. Hence the baits were replaced by 5 baits of each of the other three vaccine bait types offered to the other kudu. Again, the animals in group C did not consume these baits within 24 hours. Interesting, together with one animal in group B (apple-flavoured corn meal bait), a kudu in group C (no bait consumption) tested positive in ELISA 62 days post bait distribution. Two animals in group D already tested positive in ELISA on the day, the baits were offered. None of the other animals seroconverted after baits had been offered to the animals

Table 17: Number of baits removed after selected periods in pens where vaccine baits were offered free-of-choice (h – hours

Group	Animals (n)	Bait type	Number of baits present			
			0h	3h	8h	24h
A	2	I	10	1*	-	-
B	3	II	15	11	5	1
C	2	III	10	10	10	10
D	3	IV	15	1	1	1

* - bait left was found on the ground and removed from the pen

Bait type: I – original camel thorn tree pods, II – apple-flavoured corn meal, III – ground camel thorn tree pods mixed with gelatine (dipped), and IV - ground camel thorn tree pods mixed with gelatine (dipped)

Finally, during the first two days that 5 apple-flavoured corn meal baits and 5 baits with ground camel thorn tree pods mixed with gelatine were distributed over 3 acacia trees, all baits were located and consumed by the free-roaming male kudu. On a third occasion, none of the baits was taken and after several days the dehydrated baits were removed. (Figure 18 lower panels)

The serological results of the vaccine bait trials are summarized in table 18.

Table 18: Results of blood samples taken from the animals that were offered vaccine baits free-of-choice

Animal	Group	Bait type	B0		B1 (39d)		B2 (62d)	
			ELISA	RFFIT	ELISA	RFFIT	ELISA	RFFIT
1-1	A	I	-	-	-	-	-	-
1-4	A	I	-	-	-	-	-	-
1-7	B	II	-	-	-	-	-	-
1-8	B	II	-	-	-	-	+	-
1-9	B	II	-	-	-	-	-	-
1-10	C	III	-	-	-	-	+	-
1-14	C	III	-	-	-	-	-	-
1-17	D	IV	-	-	-	-	-	-
1-19	D	IV	+	-	+	-	+	-
1-20	D	IV	+	-	+	-	+	-

seropositive (+) means >70% inhibition (ELISA) and >0.5 IU/ml (RFFIT; 100% neutralization).

Bait type: I – original camel thorn tree pods, II – apple-flavoured corn meal, III – ground camel thorn tree pods mixed with gelatine (dipped), and IV - ground camel thorn tree pods mixed with gelatine (dipped)

5. Discussion

Rabies in kudu antelope in Namibia, although well documented by now, remains a unique phenomenon, especially with regard to the fact that the disease keeps recurring in epidemic waves – to the extent that it can now be regarded as endemic - associated with large scale losses within a short period of time. There now also exists sufficient evidence, that the manifestation of the disease in kudu no longer fits the pattern of conventional sylvatic rabies cycles in Namibia, with the jackal acting as reservoir of RABV with occasional spill-over into other animal populations, where it remains a dead-end infection. Many questions, especially concerning the apparent exceptional susceptibility of this species and possible routes of infection and transmission, other than bites from infected animals, remain unanswered and need to be investigated further.

Notwithstanding all the unanswered questions, efforts should be focussed on perfecting a method of oral vaccination of kudu and other affected antelope like eland, which is effective, practical and safe.

With regard to results of the experimental studies related to the first epidemic 1977 – 1983, the following should be noted:

The titres of RABV obtained in the first study are possibly not a true indication of the actual titres since the samples had been exposed to room temperature during the period from collection to titration. The period also varied from 4 – 16 days. Nevertheless, the results shown in Table 8 clearly illustrate the high concentration of virus in the saliva of kudu that died of rabies. The titres of RABV in the saliva of dogs can vary from a trace to 10^5 mouse LD50/ml (Vaughn et al.,1965). In most dogs, titres between 10^2 and 10^3 mouse LD50/ml are found. The concentration of virus in the saliva of only a small percentage of experimentally infected foxes was higher than $10^{3,5}$ mouse LD50/ml. In skunks, the titre of virus in the saliva was usually equal to or lower than the titre of virus in the salivary glands.

In the 3 kudu tested, the titres of RABV in the saliva were higher than those in the salivary glands (Table 8). In kudu 570/80, it was $10^{2,9}$ mouse LD50/ml higher than that in the salivary glands. The high titre of virus in the saliva is an indication of active excretion of virus. It possibly also indicates virus replication in tissues other than salivary glands. The high titres of virus in the saliva and the grooming habits of kudu provide ample opportunity for virus transmission from kudu to kudu.

Virus titres and strains play an important role in non-bite transmission of rabies. In this study it was shown that, in some instances at least, the susceptibility of species involved is also of importance. Attempts to infect cattle (Table 9) with infected saliva or mice (Table 11) with infected brain, failed, whereas 2 out of 4 kudu were readily infected with infected saliva. They died after 21 and 33 days, respectively, after oral and nasal instillation. Kudu were also readily infected with HEP Flury virus. Four out of 6 kudu exposed reacted by production of virus-neutralizing antibodies (Table 9), whereas none of 6 cattle developed antibodies to rabies virus after oral and nasal exposure to HEP Flury strain. These results indicate that kudu are highly susceptible to oral and /or nasal infection and that vaccination via this route may be possible.

Test Results: The diagnosis of rabies by FAT is as simple in kudu as it is in other species. No difference could be observed in respect of brilliance of fluorescence or the morphology and distribution of inclusion bodies. The results obtained with the FAT agreed with those obtained by histological examination. Mice and guinea pigs inoculated with the kudu isolate reacted in the same way as with isolates from other species. In virus neutralization tests, virus from kudu could not be distinguished from virus isolated from other species (Barnard et al., 1982).

Since 1977 Rabies in kudu in Namibia no longer can be described as a “dead-end” infection in a “spill-over” population in terms of the traditional sylvatic rabies cycle (Section 4.1.1, Section 4.1.2, Fig. 20, Table 20, Table 21, Table 22). During the first epidemic between 1977 and 1984, rabies was confirmed in 96 jackal and there is some indication that peaks in rabies cases in kudu coincided with peaks in jackal cases (1980). During the interepidemic period 1985 – 2000, this figure was 298 jackals and since the start of the second epidemic, only 146 cases. In comparison to this manifestation, there is a strong correlation between the peaks in dog rabies and peaks in spill-over populations with regard to the dog rabies cycle between 1977 and 2017 (Figure 20, Figure 21).

The fact that recurrent large-scale epidemics of rabies only occur in kudu in Namibia and in no other population of the Greater Kudu in southern and east Africa remains unexplained. There are a number of possible reasons or causes for this unique phenomenon. The following hypothetical scenarios should be considered: Firstly, markedly increased inherent susceptibility of kudu to RABV, secondly immunosuppression, either permanent or temporary, thirdly a separate non-bite transmission cycle.

Species related susceptibility: The large numbers of kudu that are affected by rabies and succumb to the disease may indicate that kudu could be inherently very susceptible to RABV. The other antelope species affected to a similar extent is the eland (*Taurotragus oryx).* Once again, the high incidence of rabies in eland seems to be unique to Namibia. Both kudu and eland are spiral-horned tragelaphine species and both are predominantly browsers. Kudu and eland are closely related. An apparent increased susceptibility of kudu antelope to other infectious diseases like anthrax has also been documented (Oberem & Oberem, 2012). Moreover, kudu and African buffalo are regarded as the most important maintenance hosts of bovine tuberculosis (Oberem & Oberem, 2012).

Immune compromise or immunosuppression: In the course of informal discussions by veterinarians associated with the project, the possibility that kudu antelope may suffer from immunodeficiency or immunosuppression was raised. Generally, stress is regarded as the most prevalent cause of suppression of the immune system. Capture, translocation, overcrowding, confinement, nutritional deficiencies and captivity are clearly associated with stress. It is well documented that species like African buffalo, roan and sable antelope are susceptible to stress and will readily succumb to infectious diseases like theileriosis and babesiosis (Oberem & Oberem, 2012). Except for occasional capture and translocation, free-ranging kudu in Namibia are, however, usually not subject to the above-mentioned factors and therefore it seems unlikely that stress-induced immunosuppression is a relevant cause of the abnormal occurrence of rabies in Namibian kudu. This is further supported by the observations and results of the parenteral vaccination studies described in this thesis (Table 14).

Nine calves were born in captivity from experimental animals during January and February 2016 in the research facility. These cows became pregnant during April and May of the previous year. All the stress associated with the capture, translocation, captivity, and repeated immobilization during captivity, as well as various procedures such as vaccination and sampling of the animals, did not lead to a single abortion by any of the female animals being noticed during the course of the study. Even if the possibility of early foetal absorption in some animals is considered, it appears that kudu are rather refractory to stress, although stress factors such as poor nutrition, injury, and disturbance have been quoted as causes of abortion in wildlife in southern Africa (Oberem & Oberem, 2012).

The possibility of a potential impact of immunosuppressive infectious diseases remains. A group of researchers demonstrated antibodies against a pestivirus in a significant number of kudu (Depner et al., 1991), confirming exposure of these antelopes to the virus. Although there is currently no evidence of any immunosuppressive effect of such a pestivirus infection in kudu, this cannot be disregarded completely as a possible cause of immunosuppression, which could even be only temporary, thus possibly increasing the susceptibility of the antelope to RABV. In future, all serological surveys of free-ranging kudu in Namibia should also cover this pestivirus.

Transmission: There can be no doubt that rabies in kudu in Namibia no longer fits the pattern of traditional sylvatic transmission cycles. The mode of transmission and spread of RABV is the determining factor that will explain the large-scale epidemics or endemnicity of rabies in kudu. The possibility of horizontal transmission of rabies between kudu was raised and discussed right from the initial outbreak and this topic is currently still intensively and controversially discussed. Although phylogenetic work of Scott and co-workers seems to provide evidence of horizontal transmission (Scott at al., 2013) the results contradict earlier findings by Mansfield and co-workers (Mansfield et al., 2006) and also contradict most recent phylogenetic work done by Müller and co-workers (unpublished). These findings suggest that jackal and kudu may form part of the same epidemiological cycle of rabies in Namibian wildlife, although this cycle seems to differ from the traditional one. There is reason to believe that the selective choice of RABV isolates, both by species and geographical origin by Scott and co-workers might have biased the outcome of the full genome sequencing and resulting conclusion at that time. Whole genome sequencing of RABV isolates from various species in Namibia has confirmed that RABV infection in kudu originated from black-backed jackal in all the isolates from kudu samples. Epidemiological data, however, indicate that the peaks of rabies cases in kudu do not coincide with the rabies peaks in the jackal (Figure 7). The infectious bite by this common vector therefore cannot be the only mode of transmission to result in the large numbers of rabies cases in kudu in Namibia.

Personal observations of the current outbreak in the central regions of Namibia indicate that at the start of an outbreak mainly male animals are affected, followed later by females. This is also borne out by laboratory results of animals which tested FAT positive. There is also a strong indication that this pattern may be related to the rutting season of these antelope.

In the absence of any evidence of other forms of transmission, serious consideration needs to be given to horizontal spread and transmission between kudu as a result of contamination with infected saliva, through some form of non-bite contact, either direct or indirect. Although previous and current studies have not yet produced conclusive evidence to prove horizontal

transmission they have produced overwhelming facts to suggest that this form of transmission could possibly play the key role in intermittent outbreaks of rabies in kudu. It also appears prudent to claim that rabies in kudu is now endemic rather than manifesting itself in an epidemic form. Sampling of animals for this project on the day of capture has shown the presence of anti RABV antibodies in free-ranging kudu during inter-epidemic periods may point to the remote possibility that RABV could be maintained within the kudu population. This concept requires further investigation.

Further circumstantial evidence of the possible existence of non-bite transmission of rabies in other species was provided by clinical cases of rabies in lion (*Panthera leo).* When the first epidemic of rabies occurred in kudu in the Etosha National Park during 1983-1984, the first cases of lions contracting rabies in Etosha were also recorded. All four reports of rabid lions were from the eastern sector of Etosha, where the highest density of kudu occurred, and it seems likely that these and other lions became infected, when they hunted rabid kudu, which may have been relatively easy prey. The typical hunting method employed by lion, i.e. strangling or suffocating prey, would bring the mouth and eyes of the lion into direct contact with infected saliva. Contamination of the mucous membranes with infected saliva could have resulted in non-bite transmission of the RABV from kudu to the lion (Berry 1993).

The same mechanism has been postulated to serve as a route of transmission of bovine tuberculosis from infected African buffalo to lion in the Kruger National Park in South Africa (Oberem & Oberem, 2012).

Previous and current vaccine trials were aimed at establishing the proof of principle for a possible oral vaccination of kudu against rabies. Expanding the concept of oral vaccination against rabies to kudu seems to be a feasible approach considering that commercial products for oral immunization of ruminants already exist; e.g. for immunizing calves in controlling diarrhea caused by bovine rotavirus and coronavirus (Calf-Guard®, Zoetis). However, mucosal immunization through oral delivery is often compromised by antigen degradation in the stomach, especially considering the complex gastro-intestinal tracts of larger ruminants (Shewen et al., 2009). Therefore, to facilitate antigen uptake at Peyer's patches of the intestine, additional substances are often added to oral vaccine formulations protecting the antigen against enzymatic and proteolytic degradation. However, the gut is not the only site with mucosa-associated lymphoid tissue (MALT) containing lymphoid follicles and M-cells. MALT can also be found in nasal and oral cavities, whereby tonsils form a major component in the latter. Recent studies indicated that the palatine tonsils are a major site of vaccine uptake for oral vaccination of meso-carnivores against rabies (Vos et al., in press). Palatine tonsils are also present in ruminants like cattle, sheep and goats (Casteleyn et al., 2011).

Experimental studies in wild ruminants like white-tailed deer (*Odocoileus virginianus*) showed that direct oral administration of BCG-vaccine targeting the pharyngeal lymphoid tissue was able to induce an immune response against bovine tuberculosis (Nol et al., 2008). Also, oral administration of heat-inactivated *Mycobacterium bovis* to red deer (*Cervus elaphus*) induced some level of protection against bovine tuberculosis (Lopez et al., 2016). In this study, evidence was found that also in kudu a protective immune response could be induced after direct oral administration, since 3 of 10 animals survived a severe rabies challenge infection whereby all 4 control animals that had received the same challenge dose succumbed to rabies between 12 and 16 days post infection. Interestingly, none of these 3 animals that had survived had shown detectable levels of antibodies in both ELISA, and

RFFIT assays. The vaccine strain SPBN GASGAS has been shown efficacious in several animal species like raccoon (*Procyon lotor*) and small Indian mongoose (*Herpestes auropunctatus*) (Blanton et al., 2007; Vos et al., 2013). It is therefore suggested that kudu are rather refractory to this route of rabies vaccine administration as has been observed in other species like the striped skunk (*Mephitis mephitis*) (Vos et al., in press). It needs to be investigated, how the effectiveness of vaccine uptake might be improved; for example, by increasing vaccine titers or adding muco-adhesive substances. Finally, it must be mentioned that the vaccine virus was evenly distributed in the oral cavity of the kudu using a needleless syringe simulating release of vaccine when the animal chewed on a vaccine sachet and the palatine tonsillar crypts as potential uptake site were not specifically targeted.

Unfortunately, no detectable levels of rabies antibodies were found in the animals after vaccine bait consumption. The principal purpose of this study was bait uptake and not efficient vaccine delivery; hence, no challenge infection was originally incorporated in the approved study protocol for this part of the study. Based on the results of the direct oral administration study, in which none of the three survivors developed a detectable antibody response, it cannot be ruled out that also several kudu that consumed the vaccine baits would have survived a challenge infection.

A major unexpected problem encountered during this study was the performance of the assays used, although the results obtained with the animals vaccinated by the parenteral route showed for both serological assays a perfect association between seroconversion and protection. However, for the animals that received the vaccine by the oral route, the serological results were difficult to interpret. Also, diagnosis of rabies virus antigen in the brain using the gold standard, the FAT, was not always without difficulties. Two of 11 animals that were found dead or were euthanized after showing clinical signs tested negative in FAT. However, both these brain samples, which were subsequently tested by rt-PCR, were positive with this method. These findings underscore that diagnostic assays validated (specificity and sensitivity) for samples from certain species are not automatically suitable for samples from other species, as was recently shown for serology (Moore et al., 2017).

The experimental baits were well accepted by the kudu, also when distributed by the suggested system, i.e. piercing the baits on thorns of acacia trees, a natural food source for kudu. However, differences were clearly visible between individual animals. Bait uptake was lower in pen 2 than in pen 1 during the first screening study. However, the amount of food intake of the former group of animals was generally low. Also, both animals in group C did not consume any of the baits containing the vaccine sachet, irrespective of the bait type. Even the camel thorn tree pods were not consumed by these animals, while all bait pieces were eaten rapidly by the animals in the other groups. Under field conditions, the baits were well accepted, but more intensified studies with the different baits need to be carried out to identify the optimal bait.

The third component of the oral vaccination approach concerns a suitable bait distribution system. In Europe and North America, oral rabies vaccine baits are distributed predominantly by airplane using a pre-determined bait density per km². The distance between individual bait drops is fixed, as is the distance between the flight lines (Müller et al., 2012). In certain areas, like heavily populated regions, baits cannot be distributed by plane and are therefore distributed by hand. Sometimes, new strategies like clustered

baiting, e.g. bait stations, are used (Boulanger et al., 2008). These techniques are also applied for wild boar (*Sus scrofa*), a species with a similar social structure as kudu (Ballesteros et al., 2011; Rossi et al., 2015). Unfortunately, most of these bait distribution systems are not suitable for targeting kudu in Namibia.

Hence, a system was tested that may considerably reduce bait depletion by non-target species including insects and rodents. Also, the system to be developed should reduce exposure to high temperatures and direct sun light, which can be detrimental for the vaccine baits; both for the bait matrix (melting) and the vaccine.

Piercing baits on thorns at a certain minimum height within the acacia trees favored by kudu could offer a suitable solution to bait depletion by non-target species, including smaller antelopes (browsers). A similar distribution system by placing baits at a certain height in canopies of trees to avoid bait competition by ground-dwelling animals and over-flying birds has been suggested for baiting gorillas against Ebola (Dolgin, 2008). Furthermore, such a system reduces the thermal impact on the bait and the vaccine, while the canopy of the trees/shrubs, in which the baits are placed, protects them from direct sunlight.

Placing the baits not on the ground into the grass vegetation, but in places exposed to air movement, increases heat loss from the bait to the air by convection.

Video material from the CCTV cameras installed in two pens was particularly valuable for the bait acceptance study. Video recordings during the bait uptake studies clearly showed that animals would always first take camelthorn pods, before they accepted other types of bait. From the recordings, it also appears that some baits were taken during the day, while the rest was consumed during the night. When other feed like lucerne hay, to which the animals had become accustomed, was available nearby, the animals first fed on this, before turning to the baits suspended in the bushes.

It also became evident that the kudu accepted the bait more readily with repeated exposure to the bait. Once the animals got to know the bait, they would no longer hesitate before taking it. Kudu are most active – including browsing – late in the afternoon and early in the night, as well as early in the morning, resting during the heat of the day and mostly during the night, although single animals can be seen feeding during the night. These feeding habits were confirmed by recordings from the surveillance cameras in the boma.

During and shortly after the rainy season in Namibia (January to June), an abundance of food is usually available to domestic and wild ruminants, except in years when the rainfall is significantly below average or when drought conditions prevail. Food availability starts to decline during the cold dry season (July – September) and may become critical during the hot dry season. Fruit or seed pods of camelthorn trees (*Acacia erioloba*), which are nutritious and eagerly eaten by many herbivores, particularly kudu and eland, ripen and become available after the rainy season. The pods from camelthorn trees, although very tasty and nutritious, tend to contain prussic acid (hydrocyanic acid) under certain conditions, which can give rise to poisoning. (Mannheimer et al., 2012). Care must therefore be taken when using these fruits as baits.

Kudu regularly drink water from watering points for domestic livestock during the cold dry season and the hot dry season and also from open water during and shortly after the rainy season.

Taking all of the above into account and considering that the vaccine contained in bait destined for uptake by kudu and the bait itself need to be protected against heat, sunlight, desiccation and rain, the following bait distribution protocols may be feasible:

1. Time of the year: cold dry season. Food becomes scarce and ambient temperatures decrease. The animals start to visit watering points and open water, if available, more often. This season would therefore be suitable for bait distribution.
2. Time of day: Afternoon. The bait should be distributed in the early afternoon, so that it is available during the late afternoon, night and early morning. Temperatures will be low and the timing will fit the feeding habits of the animals. Chacma baboons that may compete for the baits in areas where the animals occur, are not active at this time of the day.
3. Locations: The most suitable locations are probably bushes and trees normally browsed by kudu in the vicinity of water points. The baits should be hung high enough above the ground to make them accessible to kudu but prevent smaller antelope like springbuck (*Antidorcas marsupialis)* from removing them. This will, however, not allow kudu calves younger than three months to reach the bait. However, they may be protected through passive immunity obtained from their dams until they are tall enough to reach the baits. At a convenient height for kudu, the bait will be accessible to the larger eland antelope (*Taurotragus oryx),* - themselves browsers- in those areas where they occur. Provided that eland also find the bait palatable, this would constitute serious competition. The same would apply to cattle, but cattle could be removed from the area for the duration of the vaccination campaign. Since eland are also very susceptible to rabies, it may actually be an advantage, if they are vaccinated using the same bait intended for kudu, although at this stage it is speculation that these antelope could also be immunized against rabies via the oral route. Changes in the exposure of the selected bushes to the sun during the course of the day also need to be considered to minimize the exposure of the bait to heat and direct sunlight.
4. Type of the bait: In studies where camelthorn tree pods were offered together with the other three types of bait, the animals first ate the camelthorn tree pods. This might therefore be the bait of choice, although eventually all types of bait were consumed. Another worthwhile consideration may be to dip the individual pieces of bait in molasses syrup to enhance the palatability, before suspending them in the bushes.
5. Pre-baiting and luring: Domestic livestock and game are attracted to rock salt when it is provided by the farmers as supplement and kudu will lick on it very intensely. If rock salt is provided in the vicinity of the trees that are selected for baiting a few months before, this may help to attract the kudu to the “vaccination area”. Other forms of commercial supplements that might serve the same purpose include game lick blocks. From the experience gained from the bait studies, it may also make sense to “pre-bait” with placebo baits /or camelthorn tree pods for a number of days, before distributing the vaccine baits, as this may reduce the uptake time, once the animals have become accustomed to the baits.
6. Duration and frequency of baiting: The greatest challenge that an oral vaccination protocol for kudu antelope will face, is to ensure that the large majority of the animals have the opportunity to eat the bait and thus become exposed to the vaccine. The studies have shown, however, that a single dominant animal may

repeatedly take all the bait provided. Although no data are available yet, it can be reasonably assumed that repeated uptake of multiple doses of vaccine will enhance the immune response and increase the possibility of achieving protective immunity. Therefore, it makes sense to provide sufficient quantities of bait in different locations repeatedly over a period of time. A significant factor with regard to oral vaccination in herbivores is the fact that they chew their food extensively before swallowing. This will keep any vaccine in contact with the mucous membranes for extended periods. Repeated exposure to baits should therefore significantly improve the uptake of sufficient quantities of vaccine. Another important factor in the feasibility of any oral vaccination protocol or program will undoubtedly be the cost of the vaccine and bait. This issue should be addressed in future vaccine studies in these antelope.

7. The successful implementation of any oral vaccination program for kudu antelope against rabies will certainly face many practical challenges. Further research may provide some solutions, but ultimately it will be the dedication and practical skills of the farmers and their detailed knowledge of the terrain and the dynamics of the kudu population and other game populations on the farms, that will determine success or failure of a vaccination protocol. Although basic principles regarding bait distribution can be identified, individual protocols will have to be tailored to the prevailing conditions in each case and on every farm. Any vaccination campaign should be followed up by serological screening of hunted or captured animals.

Since the RABV isolated from kudu is closely related to RABV whole genome sequences obtained from virus isolated from black-backed jackals (Freuling, pers. communication), it will be prudent to establish the possibility of orally vaccinating jackal against rabies, using existing vaccines and a suitable bait, independent from further studies in kudu. Currently, plans are being drafted to implement a pilot study to address this problem in Namibia in the near future.

Possible causes for the low percentage of seroconversion and low survival rate after challenge following DOA of the SPBN GASGAS vaccine (Tables 13 – 15) were considered. A possible shortcoming in the administration method could have been the cause. The animals had to be immobilized for the procedure using thiafentanil and azaparone. This resulted in a temporary partial paralysis of the tongue and increased salivation. This in turn probably caused that significant volumes of the vaccine were lost from the oral cavity and insufficient amounts of vaccine may thus have come into contact with the oral mucous membranes. Moreover, the contact period may have been too short. Greater care should therefore be taken by replacing the tongue and holding the head of the animal up for some time. The problem may also be overcome when animals have the chance to extensively chew the vaccine-containing bait, as observed on camera during the bait trials.

None of the calves born during captivity to vaccinated female kudu were subjected to any serological testing to determine the possible presence of maternal anti-rabies antibodies and hence passive immunity as a result of vaccination and challenge infection. The decision taken at the time not to do so was based on the cost implications since this activity had not been budgeted for. Consideration was also given to animal welfare aspects associated with the immobilization of these young animals. Due to the usually short duration of passive immunity, such animals would have to be immobilized between 8 – 12 weeks of age. In retrospect, however, this must be considered as a missed opportunity.

As far as epidemiological studies are concerned, no attempts have been made so far to investigate and interpret any possible correlation between major events like rainfall fluctuations and the cyclic occurrence of rabies in kudu. This should be considered in future epidemiological investigations.

Similarly, the reason/s for the negative FAT results (chapter 4.1.2) have not been determined to date. Possible reasons include sample quality, test protocols and other causes of death of these animals. Further investigations, including necropsies, in this regard need to be considered urgently.

6. Conclusion and Outlook

In conclusion, kudu antelope can be infected and also vaccinated by the oral route and thus protected against a subsequent rabies infection, although it seems that they are rather refractory to this route of vaccine administration. Hence, further studies need to be conducted to optimize oral vaccine delivery for example determination of minimum effective dose for kudu, multiple (interval) application of vaccine containing bait, evaluation of the possible use of immune enhancers (adjuvants) and the testing of recombinant rabies virus vaccines using more replication competent viruses as backbone to enhance vaccine uptake and improve immune response following oral application.

Several candidate baits have been identified that were readily taken up by captive and free-living kudu. Finally, a bait distribution system has been suggested that maximizes uptake by the target population while minimizing uptake by non-target species. However, this system has to be further refined to assure high vaccination coverage among kudu and further investigation into potential bait competitors needs to be carried out. The ultimate system will have to be suited for large scale field use.

The epidemiology of rabies in kudu should be further studied to conclusively prove or disprove horizontal non-bite transmission of rabies in these antelope and to determine the potential role of the black-backed jackal in the transmission cycle. Rabies in kudu should not be studied in isolation, but all efforts aimed towards finding a solution to protect kudu from rabies, should include eland, which appear to be just as much at risk as kudu.

The concept of oral vaccination will, if necessary, be extended to the black-backed jackal, the main vector in the sylvatic rabies cycle in Namibia.

Finally, genome sequencing of RABV isolates circulating in Namibia should be employed more extensively in future to shed light on the intricate inter-relationship of rabies in wildlife and domestic animal species in this country.

7. Summary

Namibia is one of the countries where both dog rabies as well as wildlife rabies commonly occur. Since 1977, the country has experienced two epidemics of rabies in kudu antelope, manifesting in recurring cycles (Figure 20), with the second epidemic persisting until today, to aa point where rabies in kudu can be regarded as endemic. This phenomenon of rabies in kudu is unique to Namibia and does not occur in any other part of natural range of this antelope species in the rest of Africa. The disease is responsible for large numbers of fatalities in the Namibian kudu population, resulting in severe economic losses to the game farming and hunting industry. There is no indication that the disease in kudu will abate any time soon. Although genetic studies of the RABV isolated from kudu suggest that the rabies virus affecting kudu may originate from jackal, epidemiological surveys, clinical observations and transmission experiments confirm that non-bite transmission of rabies in kudu, through direct or indirect contact, is possible. This route of infection may be the most important mode of transmission and spread of the disease in kudu, also explaining the serious extent of the disease once it flares up in a susceptible population.

Intra-muscular vaccination using a commercial inactivated vaccine has been shown to be effective in protecting kudu against rabies but it remains a costly method with practical limitations and no information on the duration of immunity available so far. Experiments have delivered definite proof that protective immunity against rabies can be achieved in kudu using an oral vaccine, but methods and protocols need to be improved in order to achieve protective immunity in a sufficient number of animals.

Different types of suitable bait were developed and their uptake by kudu tested successfully. It should therefore be possible to further develop this approach into an effective delivery method for the rabies vaccine.

Serological tests required to effectively monitor the immune response to rabies vaccines, validated for the use in this species, need to be developed.

Taking everything into consideration, it should be possible develop the necessary tools, methods and protocols to effectively protect kudu antelope against rabies, within the foreseeable future.

8. Zusammenfassung

Tollwut beim Groß Kudu in Namibia

Namibia ist eins derjenigen Länder, in denen sowohl Hunde-Tollwut als auch Tollwut bei Wildtieren vorkommen. Seit 1977 hat das Land zwei Seuchenzüge von Tollwut bei Kudu-Antilopen erlebt, von denen der zweite bis heute andauert. Diese Ausbrüche haben einen starken zyklischen Charakter. Das Vorkommen von Tollwut beim Großen Kudu in Namibia ist einzigartig und kommt sonst nirgendwo im restlichen Verbreitungsgebiet dieser Antilopen in Afrika vor. Tollwut ist für den Tod einer großen Anzahl von Tieren verantwortlich, was wiederum einen hohen finanziellen Verlust für Wildfarmen und die Jagd zur Folge hat. Es gibt im Augenblick keinerlei Anzeichen dafür, dass dieses Phänomen in absehbarer Zukunft zu Ende gehen wird. Obwohl genetische Untersuchungen an Tollwutvirus Isolaten von Kudus darauf hindeuten, dass das Virus, welches die Seuche bei diesen Antilopen verursacht, vom Schakal stammt, haben epidemiologische Untersuchungen, klinische Beobachtungen und Übertragungsversuche bestätigt, dass zusätzlich zum Biss durch ein infiziertes Tier, eine Ansteckung und Verbreitung über die Schleimhäute durch direkten oder indirekten Kontakt möglich sind. Es wird daher angenommen, dass dieser Infektionsmodus die Hauptübertragung bei Kudus darstellt, was auch die hohen Verlustzahlen erklären könnte.

Die intramuskulare Impfung von Kudus mit einem inaktivierten kommerziellen Impfstoff hat sich als wirksam erwiesen. Obwohl Kudus auf diese Art und Weise erfolgreich gegen Tollwut geschützt werden können, bleibt diese Methode kostspielig und aufwändig. Außerdem gibt es noch keine Daten über die Dauer der Immunität. Experimente haben jedoch gezeigt, dass eine ausreichende Schutzimpfung von Kudus mit Hilfe einer oralen Vakzine möglich ist. Um einen größeren Anteil der Kudu-Population impfen zu können, muss diese Methode jedoch noch verbessert werden.

Verschiedene Ködersorten wurden entwickelt und ihre Aufnahme durch Kudus erprobt. Es sollte daher möglich sein, eine erfolgreiche orale Impfmethode auf Köderbasis zu entwickeln.

Um die Immunreaktion der Kudus auf eine Tollwutimpfung effizient überwachen zu können, bedarf es serologischer Testmethoden, welche für diese Tierart validiert sind. Diese müssen etabliert werden.

Nach dem jetzigen Wissenstand sollte es möglich sein, in absehbarer Zukunft die Entwicklung einer praktikablen und effizienten oralen Impfmethode von Kudus gegen Tollwut erfolgreich abzuschließen.

Um die Fragen beantworten zu können, warum ausgerechnet Kudu-Antilopen so hoch empfindlich für das Tollwutvirus sind und warum sich das Vorkommen dieser Seuche bei dieser Antilopenart auf Namibia beschränkt, bedarf es weiterer intensiver Forschung. Im Rahmen solcher Bemühungen müssten die Epidemiologie der Seuche, sowie Biologie, Physiologie, und Populationsdynamik der Kudu-Antilope in diesem Teil Afrikas untersucht werden.

9. Addendum

Table 19: Confirmed rabies cases in all species 1977 - 1986

Species	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986
Cattle	10	23	32	44	60	131	98	35	66	23
Dogs	18	14	33	23	25	21	47	31	37	28
Goats	-	2	-	3	3	2	2	8	2	4
Sheep	-	1	-	1	-	1	-	-	1	1
Equines	-	1	-	1	-	1	1	3	-	-
Cats	3	4	5	-	4	1	-	1	2	6
Other domestic	-	-	-	-	-	-	-	4	-	2
Subtotal domestic	31	45	70	72	92	157	148	82	108	64
Kudu	2	28	37	82	53	64	90	13	8	2
Jackal	5	8	11	23	3	18	11	11	19	4
Eland	-	-	-	2	1	6	6	1	1	1
Honey badger	1	2	-	1	1	3	-	1	1	-
Bat eared fox	2	4	2	3	1	3	-	3	3	1
Suricate& mongoose	-	-	-	-	1	2	-	2	2	-
Other wildlife	3	12		4	-	3	5	2	9	1
Subtotal wildlife	13	44	51	115	60	99	112	33	43	9
Total	43	89	121	187	152	256	260	115	151	73

Sources: Epidemiology Section DVS; Schneider, 1985

Table 20: Confirmed rabies cases in all species 1987 – 2000

Species	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000
Cattle	59	82	81	58	41	75	30	40	78	59	21	53	33	27
Dogs	34	30	51	21	20	32	18	12	29	27	34	37	39	38
Goats	3	8	11	11	5	4	7	6	13	5	3	4	8	9
Sheep	2	4	4	6	3	9	2	5	3	1	-	4	3	1
Equines	-	2	4	-	-	3	-	-	2	2	2	1	2	1
Cats	5	5	9	2	3	4	2	2	4	4	3	3	3	6
Other domestic	1	-	1	-	-	-	-	1	-	-	-	-	-	1
Subtotal domestic	104	131	161	98	72	127	59	66	129	98	63	102	88	83
Kudu	1	-	2	1	-	1	-	-	-	-	-	-	11	1
Jackal total	16	34	13	11	20	29	15	13	39	31	11	21	21	9
Jackal Etosha	6	8	-	2	6	9	1	-	7	14	1	4	1	1
Eland	-	-	-	-	-	-	-	-	-	2	-	1	1	1
Honey Badger	1	1	-	1	-	3	-	-	1	1	-	2	1	1
Bat eared fox	5	6	1	2	7	1	1	5	2	7	1	1	3	4
Mongoose	2	-	-	1	-		1	-	1	-	-	-	1	-
Other wildlife	1	2	6	2	1	1	-	2	4	2	4	3	1	-
Subtotal wildlife	32	43	22	18	28	35	17	20	47	43	16	28	39	16
Total	**137**	**174**	**183**	**116**	**100**	**162**	**76**	**86**	**176**	**141**	**79**	**130**	**127**	**99**

Table 21: Confirmed rabies cases in all species 2001 - 2017

Species	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
Cattle	42	48	53	43	69	51	68	30	38	103	69	81	115	101	106	77	57
Dogs	41	39	47	52	70	54	42	45	68	140	101	113	102	84	94	101	59
Goats	10	16	21	10	21	15	10	2	9	15	14	14	23	20	25	20	20
Sheep	2	-	-	2	3	2	1	1	1	-	3	4	4	2	2	-	1
Equines	1	5	3		2	4	1	1	1	4	4	4	4	3	5	3	3
Cats	9	9	8	14	7	15	11	2	8	13	9	18	8	15	**4**	**4**	**4**
Other domestic	1	-	-	1	1	4	-	3	-	-	1	-	1	3	2	2	-
Total domestic	106	117	132	122	173	145	133	84	125	275	201	234	257	228	238	207	144
Kudu	3	14	19	17	22	43	67	24	34	68	39	37	99	55	41	29	56
Jackal total	13	11	19	9	14	10	10	7	11	10	14	7	11	3	3	3	4
Jackal Etosha	-	1	2	1	-	1	1	2	5	5	11	2	7	-	-	1	-
Eland	-	-	1	1	1	-	-	-	3	4	2	3	6	6	9	6	5
Honey Badger	-	-	3	1	1	1	-	-	1	-	-	-	-	1	-	-	-
Bat eared fox	-	-	-	4	1	2	-	-	-	-	3	-	-	1	**1**	-	**1**
Mongoose	-	-	-	-		1	1	-	-	-	-	-	-	-	-	-	-
Other wildlife	1	-	4	1	4	2	1	-	2	5	1	2	5	2	2	5	3
Total wildlife	17	25	46	33	43	59	79	31	51	87	59	49	121	68	56	43	69
Total	**123**	**142**	**178**	**155**	**216**	**204**	**212**	**115**	**176**	**362**	**260**	**283**	**378**	**296**	**294**	**250**	**213**

Table 22: Survey Data First survey

No. of Questionnaires Distributed	No. of completed Questionnaires returned	Percentage returned	No. of farming units	Total surface area in sqkm	Est. total kudu population Before outbreak	kudu cadavers counted	Percentage of total population	Increase in kudu population		No increase in kudu population		Average kudu population density
280	143	50%	103	10 243.28	25 371	4735	18.7%	42	40.7%	61	59.3%	1/0.4 sqkm

Increase in jackal population		No. of cadavers and clinical rabies cases in jackal	Sex distribution of kudu affected by rabies					
34	33%	61	Male and female affected equally		More male animals affected		More female animals affected	
			64	62.1%	36	34.8%	3	3.1%

No. of clinical cases of rabid kudu observed	Clinical signs observed									
189	Loss of fear or tame		Paralysis		Hypersalivation		Aggression		Other	
	81	43%	78	41%	70	37%	18	9%	77	41%

Table 23: Survey Data Second Survey: Totals per region

Region	No of participating farms	Total Hectares	No of farms with game fence	No of farms with game camps	No of farms performing game counts	kudu population before outbreak	kudu population after outbreak	Estimated no of kudu mortalities	kudu mortality percentage	No of kudu cadavers counted by farmers
Erongo	6	36 280	6	6	6	1 600	640	960	-60%	447
Hardap	5	33 785	3	4	4	300	55	245	-82%	65
Khomas	14	107 311	11	12	14	2 793	1 582	1 211	-43%	368
Kunene	9	74 183	9	7	9	2 040	813	1 227	-60%	498
Omaheke	9	71 254	9	7	9	1 385	546	839	-61%	241
Oshikoto	8	21 819	6	5	6	2 396	1 571	825	-34%	486
Otjozondjupa	31	201 741	31	30	31	8 411	3 422	4 989	-59%	1 581
Otjozondjupa & Omaheke	1	10 200	1	1	1	1 000	150	850	-85%	200
Grand Total	**83**	**556 573**	**76**	**72**	**80**	**19 925**	**8 779**	**11 146**	**-56%**	**3 886**

Table 24 Survey Data second survey: individual farms

Region & Farm ID	No of participating farms	Size of farm in hectares	No of farms with game fence	No of farms with game camps	No of farms performing game counts	Kudu population before outbreak	Kudu population after outbreak	Estimated no of Kudu fatalities	Kudu fatality percentage	Number of Kudu carcasses counted by farmer
Erongo	6	36 280	6	6	6	1 600	640	960	-60%	447
1		5 000	Yes	Yes	Yes	-	120	- 120	N/A	10
2		13 500	Yes	Yes	Yes	1 000	350	650	-65%	400
3		7 980	Yes	Yes	Yes	-	-	-	N/A	4
4		Not indicated	Yes	Yes	Yes	350	120	230	-66%	27
5		9 800	Yes	Yes	Yes	-	-	-	N/A	6
6		Not indicated	Yes	Yes	Yes	250	50	200	-80%	
Hardap	5	33 785	3	4	4	300	55	245	-82%	65
7		2 333	Yes	Yes	Yes	20	10	10	-50%	6
8		4 500	No	No	No	80	-	80	-100%	3
9		6 000	Yes	Yes	Yes	100	30	70	-70%	
10		8 535	Yes	Yes	Yes	50	15	35	-70%	21
11		12 417	No	Yes	Yes	50	-	50	-100%	35
Khomas	14	107 311	11	12	14	2 793	1 582	1 211	-43%	368
12		10 000	No	Yes	Yes	-	-	-	N/A	40
13		7 542	Yes	Yes	Yes	80	80	-	0%	5
14		4 000	Yes	No	Yes	123	67	56	-46%	21
15		5 000	Yes	Yes	Yes	200	70	130	-65%	56
16		3 697	Yes	Yes	Yes	-		-	N/A	16
17		8 900	Yes	Yes	Yes	300	180	120	-40%	68

18		16 955	Yes	Yes	Yes	700	500	200	-29%	60
19		5 100	No	Yes	Yes	-	-	-	N/A	
20		7 014	Yes	Yes	Yes	450	250	200	-44%	
21		10 600	Yes	Yes	Yes	280	120	160	-57%	75
22		4 500	No	Yes	Yes	100	40	60	-60%	20
23		10 000	Yes	No	Yes	500	275	225	-45%	
24		7 059	Yes	Yes	Yes	60	-	60	-100%	1
25		6 944	Yes	Yes	Yes	-	-	-	N/A	6
Kunene	9	74 183	9	7	9	2 040	813	1 227	-60%	498
26		12 005	Yes	Yes	Yes	350	120	230	-66%	120
27		11 500	Yes	Yes	Yes	300	-	300	-100%	28
28		4 200	Yes	Yes	Yes	200	150	50	-25%	25
29		9 578	Yes	No	Yes	80	40	40	-50%	54
30		10 000	Yes	Yes	Yes	250	175	75	-30%	
31		3 300	Yes	Yes	Yes	60	8	52	-87%	20
32		6 000	Yes	Yes	Yes	200	120	80	-40%	26
33		11 800	Yes	No	Yes	250	50	200	-80%	187
34		5 800	Yes	Yes	Yes	350	150	200	-57%	38
Omaheke	9	71 254	9	7	9	1 385	546	839	-61%	241
35		3 954	Yes	Yes	Yes	40	30	10	-25%	4
36		7 279	Yes	Yes	Yes	300	150	150	-50%	16
37		15 900	Yes	Yes	Yes	-	-	-	N/A	17
38		7 600	Yes	No	Yes	350	150	200	-57%	50
39		11 473	Yes	Yes	Yes	-	-	-	N/A	20
40		8 619	Yes	Yes	Yes	250	-	250	-100%	82
41		11 000	Yes	Yes	Yes	-	-	-	N/A	18
42		Not indicated	Yes	No	Yes	300	200	100	-33%	30
43		5 429	Yes	Yes	Yes	145	16	129	-89%	4

Oshikoto	**8**	**21 819**	**6**	**5**	**6**	**2 396**	**1 571**	**825**	**-34%**	**486**
44		**1 184**	**Yes**	**Yes**	**Yes**	**70**	**25**	**45**	**-64%**	**24**
45		**Not indicated**	**Yes**	**Yes**	**Yes**	**80**	**25**	**55**	**-69%**	**15**
46		**4 128**	**Yes**	**No**	**Yes**	**400**	**400**	**-**	**0%**	**163**
47		**8 621**	**No**	**No**	**No**	**-**	**-**	**-**	**N/A**	
48		**Not indicated**	**Yes**	**Yes**	**Yes**	**230**	**-**	**230**	**-100%**	**13**
49		**Not indicated**	**Yes**	**Yes**	**Yes**	**50**	**15**	**35**	**-70%**	**21**
50		**2 800**	**No**	**No**	**No**	**-**	**-**	**-**	**N/A**	**55**
51		**5 086**	**Yes**	**Yes**	**Yes**	**1 566**	**1 106**	**460**	**-29%**	**195**
Otjozondjupa	**31**	**201 741**	**31**	**30**	**31**	**8 411**	**3 422**	**4 989**	**-59%**	**1 581**
52		**7 500**	**Yes**	**Yes**	**Yes**	**-**	**-**	**-**	**N/A**	
53		**7 000**	**Yes**	**Yes**	**Yes**	**750**	**250**	**500**	**-67%**	
54		**5 000**	**Yes**	**Yes**	**Yes**	**225**	**250**	**- 25**	**11%**	**45**
55		**5 000**	**Yes**	**Yes**	**Yes**	**220**	**15**	**205**	**-93%**	**74**
56		**11 270**	**Yes**	**No**	**Yes**	**-**	**-**	**-**	**N/A**	**30**
57		**Not indicated**	**Yes**	**Yes**	**Yes**	**106**	**34**	**72**	**-68%**	
58		**4 560**	**Yes**	**Yes**	**Yes**	**100**	**60**	**40**	**-40%**	**6**
59		**6 830**	**Yes**	**Yes**	**Yes**	**48**	**155**	**- 107**	**223%**	
60 & 61		**11 538**	**Yes**	**Yes**	**Yes**	**360**	**240**	**120**	**-33%**	**80**
62		**5 000**	**Yes**	**Yes**	**Yes**	**250**	**200**	**50**	**-20%**	**12**
63		**8 400**	**Yes**	**Yes**	**Yes**	**284**	**-**	**284**	**-100%**	**50**
64		**Not indicated**	**Yes**	**Yes**	**Yes**	**-**	**-**	**-**	**N/A**	
65		**6 000**	**Yes**	**Yes**	**Yes**	**300**	**150**	**150**	**-50%**	**140**
66		**1 300**	**Yes**	**Yes**	**Yes**	**104**	**44**	**60**	**-58%**	**30**
67		**11 650**	**Yes**	**Yes**	**Yes**	**217**	**130**	**87**	**-40%**	**37**
68		**7 115**	**Yes**	**Yes**	**Yes**	**265**	**200**	**65**	**-25%**	**30**

69		**7 160**	**Yes**	**Yes**	**Yes**	**-**	**-**	**-**	**N/A**	**3**
70		**3 200**	**Yes**	**Yes**	**Yes**	**102**	**115**	**- 13**	**13%**	**9**
71		**8 928**	**Yes**	**Yes**	**Yes**	**250**	**144**	**106**	**-42%**	**14**
72		**10 700**	**Yes**	**Yes**	**Yes**	**170**	**65**	**105**	**-62%**	
73		**9 998**	**Yes**	**Yes**	**Yes**	**240**	**160**	**80**	**-33%**	**40**
74		**9 100**	**Yes**	**Yes**	**Yes**	**300**	**220**	**80**	**-27%**	**121**
75		**6 500**	**Yes**	**Yes**	**Yes**	**400**	**100**	**300**	**-75%**	**70**
76		**8 400**	**Yes**	**Yes**	**Yes**	**700**	**350**	**350**	**-50%**	**312**
77		**8 325**	**Yes**	**Yes**	**Yes**	**1 600**	**-**	**1 600**	**-100%**	**54**
78		**7 083**	**Yes**	**Yes**	**Yes**	**400**	**-**	**400**	**-100%**	
79		**Not indicated**	**Yes**	**Yes**	**Yes**	**120**	**90**	**30**	**-25%**	**15**
80		**14 278**	**Yes**	**Yes**	**Yes**	**600**	**300**	**300**	**-50%**	**403**
81		**9 906**	**Yes**	**Yes**	**Yes**	**300**	**150**	**150**	**-50%**	**6**
82		**10 200**	**1**	**1**	**1**	**1 000**	**150**	**850**	**-85%**	**200**
Otjozondjupa & Omaheke	**1**	**10 200**	**Yes**	**Yes**	**Yes**	**1 000**	**150**	**850**	**-85%**	**200**
Grand Total	**83**	**556 573**	**76**	**72**	**80**	**19 925**	**8 779**	**11 146**	**-56%**	**3 886**

The farm names have been replaced by numbers in this table and the names, personal details and contact details of the farm owners have been omitted for reasons of maintaining confidentiality of data.

Table 25 Results of Monoclonal Antibody Studies of RABV from kudu isolates 1981

Animals Country	Antinucleocapsid Monoclonal Antibodies																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
	502-2	103-7	206	209-1	229-1	590-2	515-3	104-4	111-2	111-14	239-10	389-2	377-7	222-9	237-3	120	390-1	422-5
P.V.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
French Fox	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Tunisia Dogs	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-
Rwanda Dogs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Upper Volta Dog	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Madagascar Dog 1	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	+	-
Madagascar Dog 2	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-
Madagascar Dog 3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Thailand Dogs 1	+	+	+	+	+	+	+	+	+	_	+	-	+	+	+	-	+	-
Thailand Dogs 2	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	-
Thailand Dogs 3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-
Kudu 1 and 2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
Duvenhage	+	-	-	-	-	-	+	+	+	+	-	+	-	-	-	-	-	+
Lagos Bat Nigeria	+	-	-	-	-	-	-	+	+	+	+	+	-	+	-	-	-	+
Lagos Bat Central African Republic	+	-	-	-	-	-	-	+	+	+	+	+	-	+	-	-	-	+
Mokola Nigeria	+	-	-	-	-	-	-	+	+	-	+	-	-	+	+	-	-	+

10. References

Addy P. A. K. Epidemiology of rabies in Ghana. In: Kuwert E., Merieux C., Koprowski H. and Bögel K. (eds) *Rabies in the Tropics*. Berlin, Springer, 1985, pp. 497-515.

Afonso C.L. Taxonomy of the order Mononegavirales: update 2016. *Arch Virol.* 2016; **161**:2351–2360.

Afshar A. A review of non-bite transmission of rabies virus infection. *Br Vet J.* 1979; **135**: 142–148.

Amarasinghe G K, Arechiga Ceballos N. G., Banyard, A. C., Basler C. F., Bavari S., Bennett, A. J. et al. Taxonomy of the order Mononegavirales: update 2018. *Arch Virol*. 2018 [in press]

Anderson A., Shwiff S. A. The cost of canine rabies on four continents. *Transbound Emerg Dis*. 2015; **62**(4): 446–452.

Anon. Constitution of the Republic of Namibia. http://www.gov.na/documents/10181/14134/Namibia_Constitution.pdf/37b70b76-c15c-45d4-9095-b25d8b8aa0fb, accessed 23/06/2018

Anon. Ministry of Agriculture, Water and Forestry. Government Notice 180 of 2013: Declaration of Notifiable Diseases; Animal Health Act, 2011. *Government Gazette of the Republic of Namibia*. 2013; 5239: 5.

Anon. Office of the Prime Minister. Government Notice 46 of 2011: Promulgation of Animal Health Act, 2011 (Act No 1 of 2011), of the Parliament. *Government Gazette of the Republic of Namibia*. 2011; 4694: 1

Anon. Rehoboth Town Council. Regulations for the control of dogs in Municipal areas. Promulgated by Government Notice 131 of 1968. *Government Gazette of the Republic of Namibia*. 2006; 3687: 2

Anon. Republic of Namibia. Nature Conservation Ordinance 4 of 1975 as amended, OG 3469, 1975.

Ayalew Y. Analysis of 159 human rabies cases in Ethiopia. In: Kuwert E., Merieux C., Koprowski H. and Bögel K. (eds) *Rabies in the Tropics*. Berlin, Springer, 1985, pp. 481-484.

Badrane H., Tordo N. Host Switching in Lyssavirus History from the Chiroptera to the Carnivora Orders. *J Virol.* 2001; **75**(17): 8096-8104.

Ballesteros, C., Vicente, J., Morriss, G., Jockney, I., Rodriguez, O., Gortazar, C. et.al. Acceptance and palatability for domestic and wildlife hosts of baits designed to deliver a tuberculosis vaccine to wild boar piglets. *Prev Vet Med*. 2011, **98**, 198-203.

Banyard A. C., Evans J. S., Luo T. R., Fooks A. R. Lyssaviruses and bats: Emergence and Zoonotic Threat. *Viruses.* 2014; **6**(8): 2974-2990.

Banyard A. C., Hayman D., Johnson N., McElhinney L., Fooks A. R. Bats and Lyssaviruses. In: Jackson A C (ed) *Adv Virus Res*. 2011, Elsevier, Amsterdam, pp. 239-289.

Barnard B. J. H., Hassel R. H. Rabies in kudu (Tragelaphus Strepsiceros) in South West Africa/Namibia. *J S Afr Vet Assoc*. 1981; **52**(4): 309–314.

Barnard B. J. H., Hassel R. H., Geyer H. J., de Koker W. C. Non-bite transmission of rabies in kudu (Tragelaphus Strepsiceros). *Onderstepoort J Vet Res*. 1982; **49**: 191-192.

Barnes J. I., de Jager J. L. V. Economic and financial incentives for wildlife use on private land in Namibia and the implications for policy. *S Afr J Wildl Res.* 1996; *26*(2): 37-46.

Barnes J., Lange G., Nhuleipo O., Muteyauli P., Katoma P., Amupolo H. et al. (2009) Preliminary Valuation of the Wildlife Stocks in Namibia: Wildlife Asset Accounts. Ministry of Environment and Tourism Report, Windhoek, Namibia.

Berry H. H. Surveillance and control of anthrax and rabies in wild herbivores and carnivores in Namibia. *Rev Sci Tech* 1993; **12**: 137-146.

Bingham J. Canine rabies ecology in southern Africa. *Emerg Infect Dis.* 2005; **11**(9): 1337-1342.

Blanton J. D., Self J., Niezgoda M., Faber M. L., Dietzschold B., Rupprecht C. Oral vaccination of raccoons (Procyon lotor) with genetically modified rabies virus vaccines. *Vaccine*. 2007; **25**(42): 7296-7300.

Boulanger, J. R., Bigler, L.L., Curtis, P.D., Lein, D.H., Lembo, A.J. Comparison of suburban vaccine distribution strategies to control raccoon rabies. *J. Wildl. Dis.* 2008*,* **44,** 1014-1023.

Calisher C. H., Child J. E., Field H., Holmes K. V. Schountz T. Bats: important reservoir hosts of emerging viruses. *Clin. Microbiol. Rev. 2006;* **19:** 531-545.

Casteleyn C., Breugelmans S., Simoens P., Van den Broeck W. The tonsils revisited: review of the anatomical localization and histological characteristics of the tonsils of domestic 619 and laboratory animals. *Clin Dev Immunol*. 2011; **2011**:472460.

Centers for Disease Control and Prevention (CDC). The Rabies Virus; Rabies free countries; 2017.

Cluver E. Rabies in South Africa. *S Afr Med J*. 1927; **1**(10): 247-253.

Coetzer A., Anahory I., Dias P. T., Sabeta C. T., Scott T. P., Markotter W. et al. Enhanced diagnosis of rabies and molecular evidence for the transboundary spread of the disease in Mozambique. *J S Afr Vet Assoc. 2017a;* **88:** e1-e9.

Coetzer A., Coertse J., Makalo M. J., Molomo M., Markotter W., Nel L. Epidemiology of Rabies in Lesotho: The Importance of Routine Surveillance and Virus Characterization. *Trop. Med. Infect. Dis. 2017b;* **2:** 30.

Coetzer A., Sabeta C. T., Markotter W., Rupprecht C. E., Nel L. Comparison of biotinylated monoclonal and polyclonal antibodies in an evaluation of a direct rapid immunohistochemical test for the routine diagnosis of rabies in southern Africa. *PLOS Negl Trop Dis.* 2017; **8:** e3189.

Coetzer, A., Sabeta, C. T., Markotter, W., Rupprecht, C. E., Nel, L. H. Comparison of biotinylated monoclonal and polyclonal antibodies in an evaluation of a direct rapid immunohistochemical test for the routine diagnosis of rabies in southern Africa. *PLoS Negl Trop Dis.* 2014; **8**: e3189.

Conservancies Association of Namibia (CANAM). What is a Conservancy? 2018; www.canam.iway.na/Who_is_CANAM/who_is_canam.html, accessed 23/06/2018.

Courtin F., Carpenter T. E., Paskin R. D., and Chomel B. B. Temporal patterns of domestic and wildlife rabies in central Namibia stock-ranching area, 1986–1996. *Prev Vet Med.* 2000; **43**: 13-28.

Davis A. D., Rudd R. J., Bowen R. A. Effects of aerosolized rabies virus exposure on bats and mice. *J Infect Dis.* 2007*;* **195:** 1144-1150.

Dean D. J., Abelseth M. K., Athanasiu P. The fluorescence antibody test. In: Meslin F. X, Kaplan M. M., Koprowski H. (eds) *Laboratory techniques in rabies*. 4th ed. Geneva, WHO, 1996, pp. 88-93.

Depner K., Hübschle O., Liess B. Prevalence of ruminant pestivirus infections in Namibia. *Onderstepoort J Vet Res*. 1991; **58**: 107109.

Dietzschold B., Cox J. H., Schneider L. G., Wiktor T. J., Koprowski, H. Isolation and purification of a polymeric form of the glycoprotein of rabies virus. *J Gen Virol*. 1978; **40**: 131-139.

Directorate of Veterinary Services (DVS), Ministry of Agriculture Water and Forestry, Rabies data 1977 – 2018, DVS animal disease database, accessed 12/05/2018

Directorate of Veterinary Services (DVS). Rabies Control Strategy, March 2015; unpublished document

Dolgin, E. Baiting Ebola. *Scientist.* 2008, **22**, 22-22.

East, R. African Antelope Database. IUCN/SSC Antelope Specialist Group. IUCN, Gland, Switzerland and Cambridge, UK,1998. https://portals.iucn.org/library/efiles/documents/ssc-op-021.pdf, accessed 02/03/2018

Eddington A., 1895. Report of the Directorate of the Colonial Bacteriology Institute, Cape, for the year 1894. Cape of Good Hope, Department of Agriculture, 1895.

Estes R. The safari companion: A guide to watching African mammals. Chelsea Green Publishing, Vermont, 1993.

Faber M., Faber M. L., Li J., Preuss M. A., Schnell M. J., Dietzschold B. Dominance of a non-pathogenic glycoprotein gene over a pathogenic glycoprotein gene in rabies virus. *J Virol.* 2007; **81**(13): 7041-7047.

Faber M., Faber M. L., Papaneri A., Bette M., Weihe E., Dietzschold B., et al. A Single Amino Acid Change in Rabies Virus Glycoprotein Increases Virus Spread and Enhances Virus Pathogenicity. *J. Virol.* 2005; **79**(22): 14141-14148.

Faber M., Pulmanausahakul R., Hodawadekar S. S., Spitsin S., McGettigan J. P., Schnell M. J. et al. Overexpression of the rabies virus glycoprotein results in enhancement of apoptosis and antiviral immune response. *J Virol*. 2002; **76**(7): 3374-3381.

Fahrion A. S., Taylor L. H., Torres G., Müller T., Dürr S., Knopf L. et al. The Road to Dog Rabies Control and Elimination-What Keeps Us from Moving Faster? *Front Public Health*. 2017; **5**:103.

Finke S., Conzelmann K. K. Replication strategies of rabies virus. *Virus Res*. 2005; **111**(2): 120-131.

Fooks A. R., Cliquet F., Finke S., Freuling C., Hemachudha T., Mani R. S. et al. Rabies. *Nat Rev Dis Primers.* 2017;**3,** 1-19.

Freuling C. M., Eggerbauer E., Finke S., Kaiser C., Kaiser C., Kretzschmar A., et al. Efficacy of the oral rabies virus vaccine strain SPBN GASGAS in foxes and raccoon dogs. *Vaccine*. 2017; [in press]

Gschwender F. Support to De-bushing; Project description. Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH. 2017; https://www.giz.de/en/worldwide/28648.html

Hampson K., Coudeville L., Lembo T., Sambo M., Kieffer A., Attlan M. et al. Estimating the global burden of endemic canine rabies. *PLoS Negl Trop Dis*. 2015; **9**(4): e0003709.

Hassel R. H. Incidence of Rabies in kudu in South West Africa/ Namibia. *S Afr J Sci.* 1982; **78**(10): 418-421.

Hassel R. H. Progress report on the kudu rabies epidemic. State Veterinarian Windhoek, 1980. Directorate of Veterinary Services, Department of Agriculture and Nature Conservation.

Hassel R. Rabies in kudu antelope (Tragelaphus strepsiceros) in Namibia: Past and Present. In: 10th International Conference of the Southern and East African Rabies Group (SEARG) held in Maputo, Mozambique from 25-28 January 2011.

Hayman D. T. Bats as Viral Reservoirs. *Annu Rev Virol.* 2016*;* **3:** 77-99.

Hayman D. T., Bowen R. A., Cryan P. M., McCracken G. F., O'Shea T. J., Peel A. J. et al. Ecology of Zoonotic Infectious Diseases in Bats: Current Knowledge and Future Directions. *Zoonoses Public Health*. 2013; **60**(1): 2-21.

Henning M.W. *Rabies in Animal Diseases of South Africa*, 3rd ed. Pretoria. Central News Agency, 1956.

Herzenberg L. Two cases of hydrophobia. *J Med Assoc S Afr*. 1928; **2**(24): 659–661.

Hoffmann B., Freuling C. M., Wakeley P. R., Rasmussen T. B., Leech S., Fooks A. R. et al. Improved Safety for Molecular Diagnosis of Classical Rabies Viruses by Use of a TaqMan Real-Time Reverse Transcription-PCR "Double Check" Strategy. *J Clin Microbiol.* 2010; **48**(11): 3970-3978.

Hu S. C., Hsu C. L., Lee M. S., Tu Y. C., Chang J. C., Wu C. H. et al. Lyssavirus in Japanese Pipistrelle, Taiwan. *Emerg Infect Dis. 2018;* **24:** 782-785.

Hübschle O. J. B. Rabies in the kudu antelope (Tragelaphus Strepsiceros). *Rev Infect Dis.* 1988; Suppl 4: S629-S633.

Hutcheon D. Reports of the Colonial Veterinary Surgeon and Assistant Veterinary Surgeons for the year 1893. Department of Agriculture, Cape of Good Hope, 1894.

Ibrahim A. E., Eltigani A., Ali A. A. Epidemiology of rabies in Sudan. In: Kuwert E., Merieux C., Koprowski H., Bögel K. (eds) *Rabies in the Tropics*. Berlin, Springer, 1985, pp. 465–472.

Joubert D., Zimmermann I., Nuulimba N. Baseline Study of Bush Encroachment and available Management Options for the Windhoek Green Belt Landscape. Final Report. Namibian Protected Landscape Conservation Area Project (NAM-PLACE). Ministry of Environment and Tourism. 2016; http://www.landscapesnamibia.org/windhoek-green-belt/sites/default/files/resources/Baseline%20Study%20of%20Bush%20Encroachment.pdf, accessed 10/07/2018

King A. A., Meredith C. D., Thomson G. R. Canid and viverrid rabies viruses in South Africa. *Onderstepoort J Vet Res*. 1993; **60**(4): 295-299.

Koprowski, H. The mouse inoculation test, In: Kaplan, M. M., Koprowski, H. (eds.) *Laboratory techniques in rabies*. World Health Organization, Geneva, WHO, 1973, pp. 85-93.

Lawrence J. A., Foggin C. M., Norval R. A. The effects of war on the control of diseases of livestock in Rhodesia (Zimbabwe). *Vet Rec*. 1980; **107**(4): 82-85.

Lembo T., Niezgoda M., Velasco-Villa A., Cleaveland S., Ernest E., Rupprecht C. E. Evaluation of a direct, rapid immunohistochemical test for rabies diagnosis. *Emerg. Infect. Dis. 2006;* **12**(2): 310-313.

Lindsey P., Haveman P., Lines R., et al. Benefits of wildlife-based land uses on private lands in Namibia and limitations affecting their development. *Fauna and Flora International, Oryx,* 2013; 47(1), 41–53

Lopez V., Gonzalez-Barrio D., Lima-Barbero J. F., Ortiz J. A., Dominguez L., Juste R. et al. Oral administration of heat-inactivated Mycobacterium bovis reduces the response of farmed red deer to avian and bovine tuberculin. *Vet Immunol Immunopathol*. 2016; **172**: 21-25.

Lyles D. S., Kuzmin I. V. Rupprecht C. E. Rhabdoviridae. In: Knipe D. M., Howley P. M. (eds) *Fields Virology*. 6th ed., New York, Raven Press, 2013, pp. 885-922.

Mannheimer C., Marais A., Schubert S. Toxic Plants of veterinary Importance in Namibia. Ministry of Agriculture Water and Forestry; Publish Pro Windhoek, 2012.

Mansfield K., McElhinney L., Hübschle O., Mettler F., Sabeta C., Nel L. et al. A molecular epidemiological study of rabies epidemics in kudu (Tragelaphus strepsiceros) in Namibia. *BMC Vet Res*. 2006; **2**: 2.

Maseke M., Chief Veterinary Officer (CVO), Directorate of Veterinary Services, personal communications, 2017

Mendelsohn J., Jarvis A., Roberts C., Robertson T. *Atlas of Namibia: a portrait of the land and its people*. 3rd ed. Cape Town, Sunbird Publ., 2009.

Meredith C. D. Wildlife Rabies: Past and present in South Africa. *S Afr J Sci*. 1982; **78**: 411–415.

Middel K., Fehlner-Gardiner C., Pulham N., Buchanan T. Incorporating Direct Rapid Immunohistochemical Testing into Large-Scale Wildlife Rabies Surveillance. *Trop. Med. Infect. Dis. 2017;* **2:** 21.

Ministry of Health and Social Services, Namibia. Health Information System 2001 – 2007.

Mollentze N., Biek R., Streicker D. G. The role of viral evolution in rabies host shifts and emergence. *Curr Opin Virol.* 2014*;* **8C**: 68-72.

Moore S., Gilbert A., Vos A., Freuling C.M., Ellis C., Kliemt J. Rabies Virus Antibodies from Oral Vaccination as a Correlate of Protection against Lethal Infection in Wildlife. *Trop Med Infect Dis*. 2017; **2**(3): 31.

Müller T., Selhorst T., Burow J., Schameitat A., Vos A.: Cross reactive antigenicity in orally vaccinated foxes and raccoon dogs against European Bat Lyssavirus type 1 and 2. *Dev Biol* (Basel). 2006; **125**:195-204.

Müller, T., Freuling, C., Geschwendner, P., Holzhofer, E., Mürke, H., Rüdiger, H., Schuster, P. et al. SURVIS: a fully-automated aerial baiting system for the distribution of vaccine baits for wildlife. *Berl. Munch. Tierarztl. Wochenschr.,* 2012, **125,** 197-202.

Murphy F. A. Morphology and Morphogenesis. In: Baer G. M. (ed) *The Natural History of Rabies*. Vol 2., 2nd ed., New York, Academic Press, 1975, pp. 33-61.

Namibian Veterinary Council, Register of registered veterinarians, veterinary para-professions and veterinary facilities; 2018, http://www.van.org.na/section.php?secid=9&menuid=9, accessed 23/06/2018

Neitz W. O., Marais I. P. Rabies as it occurs in the Union of South Africa. In: *Eighteenth Report of the Director of Veterinary Services and Animal Industry*. Pretoria, Government Printer, 1932, pp. 71-98.

Nel L. H., Sabeta C. T., von Teichman B., Jaftha J. B., Rupprecht C. E., Bingham J. Mongoose rabies in southern Africa: a re-evaluation based on molecular epidemiology. *Virus Res*. 2005; **109**: 165-173.

Nishizono A., Yamada K., Khawplod P., Shiota S., Perera D., Matsumoto T. et al. Evaluation of an improved rapid neutralizing antibody detection test (RAPINA) for qualitative and semiquantitative detection of rabies neutralizing antibody in humans and dogs. *Vaccine*. 2012; **30**(26): 3891-3896.

Nokireki T., Tammiranta N., Kokkonen U. M., Kantala T., Gadd T. Tentative novel lyssavirus in a bat in Finland. *Transbound Emerg Dis. 2018;* **65**(3): 1–4.

Nolden T., Pfaff F., Nemitz S., Freuling C. M., Höper D., Müller T. et al. Reverse genetics in high throughput: rapid generation of complete negative strand RNA virus cDNA clones and recombinant viruses thereof. *Sci. Rep.* 2016; **6:** 23887.

Oberem P., Oberem P. A *Guide to Animal Diseases in South Africa - Game*. Briza Publ., Pretoria, South Africa, 2012.

OIE. 2018. Chapter 2.1.17, Rabies (Infection with Rabies virus and other Lyssaviruses) In: OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.01.17_RABIES.pdf, accessed 08/06/2018.

OIE. Animal Health Surveillance; Chapter 1.4. In: *OIE Terrestrial Animal Health Code*. Paris, OIE, 2017.

OIE. OIE Terrestrial Guidelines – Glossary. In: *OIE Terrestrial Animal Health Code*. Paris, OIE, 2015, pp. 1-12.

OIE. Rabies. Technical disease cards. 2018. http://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Disease_cards/RABIES_FINAL.pdf, accessed 23/06/2018

Owen-Smith N. Assessing the foraging efficiency of a large herbivore, the kudu. *S Afr J Wildl Res*. 1979; **9**: 103-110.

Owen-Smith N., Cooper S. M. Aspects of the feeding ecology of a browsing ruminant: the kudu. *S Afr J Animal Sci*. 1983; **13**: 35-38.

Owen-Smith N., Cooper S. M. Nutritional ecology of a browsing ruminant, the kudu (Tragelaphus strepsiceros), through the seasonal cycle. *J Zool*. 1989; **219**: 29-43.

Perrin M. R., Allen-Rowlandson T.S. The reproductive biology of the Greater kudu Tragelaphus strepsiceros. *Z Säugetierkunde*. 1995; **60**: 65-72.

Rossi, S., Staubach, C., Blome, S., Guberti, V., Thulke, H.H., Vos, A et al. Controlling of CSFV in European wild boar using oral vaccination: a review. *Front Microbiol.* 2015, **6:** 1141.

Rupprecht C. E., Cliquet F., Fehlner-Gardiner C., Fooks A. R., Müller T., Sabeta, C. et al. Progress in the development of a direct rapid immunohistochemical test for diagnosing rabies. *Bull Off Int Epizoot*. 2014; **3**: 87-95.

Rupprecht C. E., Xiang Z., Servat A., Franka R., Kirby J., Ertl H. C. J. Additional Progress in the Development and Application of a Direct, Rapid Immunohistochemical Test for Rabies Diagnosis. *Vet Sci. 2018;* **5**(2): pii:E59.

Rupprecht C., Kuzmin I., Meslin F. Lyssaviruses and rabies: current conundrums, concerns, contradictions and controversies. *F1000Res.* 2017; **6:** 184.

Sabeta C. T., Khaiseb S., Ngeleja-Mpelumbe C., Shumba W. Harmonization of the fluorescent antibody test (FAT) protocol for use in the SADC countries. In: Southern and Eastern African Rabies Group (SEARG) meeting, Maputo, Mozambique, 2011, pp. 1-28.

Schneider H. P. Analyse der Tiergesundheitssituation in Südwestafrika/Namibia. Dissertation, Universität Giessen, 1977.

Schneider H. P. *Animal Health and Veterinary Medicine in Namibia: a work of reference on the occurrence and epidemiology of the most important animal diseases in Namibia during the past 150 years*. Windhoek, Agrivet, 1994, pp. 89–102.

Schneider H. P. Rabies in South Western Africa/Namibia. In: Kuwert E., Merieux C., Koprowski H., Bögel K. (eds) *Rabies in the Tropics*. Berlin, Springer, 1985, pp. 520-535.

Scott T. P., Coetzer A., Nel L. H. Rabies in Namibia, more than a horrendous disease: The social, environmental and economic challenges faced. In: Sherman, W. (ed) *Handbook on Africa: Challenges and Issues of the 21st Century Chapters*. New York, Nova Science, 2016, pp. 183-210.

Scott T. P., Fischer M., Khaiseb S., Freuling C., Höper D., Hoffmann B. et al. Complete Genome and Molecular Epidemiological Data Infer the Maintenance of Rabies among kudu (Tragelaphus strepsiceros) in Namibia. *PLoS One*. 2013; **8**(3): e58739.

Scott T. P., Stylianides E., Markotter W., Nel L. Serological survey of bovine viral diarrhoea virus in Namibian and South African kudu (Tragelaphus strepsiceros) and eland (Taurotragus oryx). *J S Afr Vet Assoc*. 2013; **84**(1): a937.

Scott T., Hassel R., Nel L. H. Rabies in kudu (Tragelaphus strepsiceros). *Berl Munch Tierarztl Wochenschr.* 2012; **125**: 236–241.

Shewen P. E., Carrasco-Medina L., McBey B. A., Hodgins D. C. Challenges in mucosal 607 vaccination of cattle. *Vet Immunol Immunopathol*. 2009; **128**(1-3): 192-198.

Snyman P. S. The study and control of the vectors of rabies in South Africa. *Onderstepoort J Sci Anim Ind*. 1940; **15**:9–140.

Stuart C., Stuart, T. *Naturführer Säugetiere des südlichen Afrikas*. Kapstadt, Struik, 1999.

Swanepoel R. Rabies. In: Coetzer J. A. W., Tustin R. C. (ed) *Infectious Diseases of Livestock*. Vol. 2, 2nd ed., Cape Town, Oxford University Press, 2004, pp. 1123-1182.

Swanepoel R., Barnard B. J. H., Meredith C. D., Bishop G. C., Brückner G. K., Foggin C. M.et al. Rabies in southern Africa. *Onderstepoort J Vet Res*. 1993; **60**: 325-346.

Swanepoel R., Barnard B. J. H., Meredith C. D., Bishop G. C., Brückner G. K., King A. A. et al. Canid and viverrid rabies viruses in South Africa. *Onderstepoort J Vet Res.* 1993; **60**: 295-299.

Van Schalkwyk D. L., Hoffman L. C. *Guidelines for the harvesting and processing of wild game in Namibia*. Windhoek, Ministry of Environment and Tourism, 2016. http://scholar.sun.ac.za/handle/10019.1/99655, accessed 12/02/2018

Veterinary Association of Namibia, List of private veterinarians and private practices; http://www.van.org.na/section.php?secid=9&menuid=9, accessed 20/03/2018

Vaughn J. B., Gerhardt P., Newell K. W. Excretion of street rabies virus in the saliva of dogs. *J.A.M.A.* 1965; 193: 363 - 368

Vigilato M. A., Clavijo A., Knobl T., Silva H. M., Cosivi O., Schneider M. C. et al. Progress towards eliminating canine rabies: policies and perspectives from Latin America and the Caribbean. *Phil Trans R Soc London B Biol Sci*. 2013; **368**(1623): 20120143.

Von Maltitz L. Rabies in the northern districts of South West Africa. *J S Afr Vet Med Assoc*. 1950; **21**: 4-12.

Von Teichman B. F., Thomson G. R., Meredith C. D., Nel L. H. Molecular epidemiology of rabies virus in South Africa: evidence for two distinct virus groups. *J Gen Virol.* 1995; **76**: 73-82.

Vos A., Kretzschmar A., Ortmann S., Lojkic I., Habla C., Müller T, et al. Oral vaccination of captive small Indian mongoose (Herpestes auropunctatus) against rabies. *J Wildl Dis*. 2013; **49**(4): 1033-1036.

Wasniewski M., Guiot A. L., Schereffer J. L., Tribout L., Mähar K., Cliquet F. Evaluation of an ELISA to detect rabies antibodies in orally vaccinated foxes and raccoon dogs sampled in the field. *J Virol Methods*. 2013; **187**(2): 264-270.

Webster W. A., Casey G. A. Virus isolation in neuroblastoma cell culture. In: Meslin F. X., Kaplan M. M., Koprowski H. (eds) *Laboratory techniques in rabies*. 4th ed., 1996, Geneva, WHO, pp. 93-104.

WHO Human rabies: 2016 updates and call for data. *Wkly Epidemiol Rec*. 2017; **92**(7): 77–86.

WHO. WHO expert consultation on rabies. 3rd Report. *WHO technical report series*. 2018.

WHO. WHO expert consultation on rabies. First Report. *WHO technical report series*. 2004; **931**:1-121.

11. Index of Previous Publications

The following table contains those parts of this dissertation which have been published prior to 01.07.2019

Chapter or Section	Name of Publication	Date
Parts of: objectives, vaccine trials, results, conclusion; Figure 18	The Manga; Newsletter of the Veterinary Association of Namibia Issue 2 / 2018	August 2018
Parts of: introduction and background, materials and methods, transmissison studies, vaccination studies sampling, viruses ethical approval diagnostic assays results Figure 14, Figure 28, discussion and conclusion; tables 23, 14 and 15	Scientific REPORTS | (2018) 8:16599 | DOI:10.1038/s41598-018-34985-5 Experimental screening studies on rabies virus transmission and oral rabies vaccination of the Greater Kudu (Tragelaphus strepsiceros) Hassel et al www.nature.com/scientific reports	09.11.2018
	PLOS Neglected Tropical Diseases Ecology and epidemiology of rabies in humans, domestic animals and wildlife in Namibia, 2011-2017; Hikufe et al	16.04. 2019
Refer to chapter 1.1 and 1.2 of this dissertation: This project forms the basis of this dissertation. Therefore large parts are based on these reports	All Progress Reports as well as the Final Report: Epidemiological Survey of Rabies in Kudu in Namibia and Development of an Oral Anti-Rabies vaccine for Kudu 15. 03. 2016	15.03.2016
Parts of: Materials and methods, baits, baiting studies, results and discussion	European Journal of Wildlife Research (2018) 64:62 https://doi.org/10.1007/s10344-018-1220-z Baiting studies on oral vaccination of the greater kudu (Tragelaphus strepsiceros) against rabies; Hassel et al	Sept 2018
Summary of the whole kudu rabies project including prelimanary results and photos	Die Jägerin III/2017 page 26 - 27	July 2017
Summary of kudu rabies project including photos Fig. 14	Pirsch Jagdmagazin 03/2016 Pages 56 – 59 www.jagderleben.de	February 2016

Acknowledgments

I would like to thank the Ministry of Environment and Tourism, the Directorate of Veterinary Services of the Ministry of Agriculture, Water and Forestry and the Namibian Civil Aviation Authority for their co-operation in expediting the issuing of all required permits for the project. I am grateful to Ortwin von Gossler, Helmut Halenke, Rainer Halenke, Peter Schaepe, Hendrik Herselman and Johnnie Hamman for donating kudu, to my colleagues Hans-Otto Reuter for executing an extremely professional capture operation of the experimental animals, Mark Jago and Floris Bruwer who assisted me with the experimental work and to Peter Clausen, Peter Reinhardt, Wilhelm, Sebson, Khaleb and Severn for supervising the keeping of the animals. Thanks also go to Pauline Lindeque for assisting with the epidemiological work and Toini Malima for the administrative support rendered to the project. I would like to express my appreciation to the Namibian farmers associations and individual farmers, the Conservancies Association of Namibia (CANAM) and individual conservancies, the Namibia Professional Hunting Association (NAPHA), Environmental Investment Fund (EIF), Game Products Trust Fund (GPF), Small Grant Program, First National Bank of Foundation, Agribank of Namibia, Paratus Telecom, B2 Gold, Blaser Jagdwaffen, Dallas Safari Club, Global Supplies, Feedmaster, Afrivet, AGRA Ltd, Safari Den and Voermol Feeds for generous funding of the project. My sincere thanks also go to the Namibia Agricultural Union who commissioned and supported this project and secured the necessary funding. In addition, a big word of thanks also needs to go to Thomas Müller, Conrad Freuling and Adrian Vos for all their hard work and dedication to this project and the organization which they represent, the Friedrich- Loeffler-Institut, Isle of Riems, Germany, and IDT Biologika, Dessau-Rosslau, Germany, for their engagement and support and for committing substantial resources to the Kudu Rabies Project. Without them the implementation of this project would have been impossible. Friedrich-Loeffler-Institute and IDT Biologika as integral partners of this projects have become indispensable to the Kudu Rabies Project. Thomas Müller and Conrad Freuling also deserve my thanks for encouraging me to write this dissertation and their assistance in this regard, as well as Cornelia Hassel who assisted with the drawing of some of the maps and my brother-in-law John Quarmby for technical assistance. I am also indebted to Prof Franz Conraths who agreed to carry out the task of supervising my work. I am extremely grateful for his professional assistance. Finally, but very importantly, I would like to thank my best friend Helmut Halenke, who has taken a very active role in the implementation of this project, who mobilized the members of the Namatanga Conservancy to donate most of the kudu required for this project and who has never stopped supporting me in this endeavour.

Eigenständigkeitserklärung

Hiermit bestätige ich, dass ich die vorliegende Dissertation mit dem Titel „Rabies in Kudu Antelope in Namibia“ selbstständig angefertigt habe. Ich versichere, dass ich ausschließlich die angegebenen Quellen und Hilfen in Anspruch genommen habe. Keine entgeltliche Hilfe von Vermittlungs- bzw. Beratungsdiensten (Promotionsberater oder anderer Personen) wurde in Anspruch genommen. Niemand hat von mir mittelbar oder unmittelbar entgeltliche Leistungen für Arbeiten erhalten, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen.

Die vorliegende Arbeit wurde bisher nicht für eine Prüfung oder Promotion oder für einen ähnlichen Zweck zur Beurteilung eingereicht.

Windhoek, den 01.07, 2019

Rainer Hubertus Hassel

mbvberlin mensch und buch verlag

49,90 Euro | ISBN: 978-3-96729-008-0