Spatiotemporal analysis of rabies in South Africa, the role of black-backed jackals (*Canis mesomelas*) and aspects of its control by oral rabies vaccination

by

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“The most important factors for completing a PhD are passion, inquisitiveness, creativity, discipline, persistence and perseverance”

Prof Dr Jamal Hisham Hashim (United Nations University

DECLARATION

I, Dr Katja Koeppel hereby declare that the thesis, which I hereby submit for the degree of *Philosophiae Doctor* at University of Pretoria, Faculty of Veterinary Science is my own work and has not been submitted to any other facility prior.

Katja Koeppel

KN Koeppel
31st October 2020

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Rabies causes fatal encephalitis in all warm-blooded animals, including humans. It is thought to have entered southern Africa in 1892 with a domestic dog (*Canis lupus familiaris*) from England; however, rabies was most likely present in yellow mongoose (*Cynictis penicillata*) before that time in southern Africa. Following the spread of disease in dogs, rabies emerged in cattle, back-backed jackal (*Canis mesomelas*) and side-striped jackal (*Canis adustus*) in Namibia, Zimbabwe and northern part of Botswana and parts of North West, Limpopo and Mpumalanga provinces of South Africa. Rabies also occurs in a variety of other wildlife hosts in South Africa.

Rabies cases in all animals from 1993 to 2019 in South Africa were reviewed and analysed using a spatiotemporal cluster analysis using a discrete Poisson space-time probability model on monthly aggregated cases. Dog and livestock density were used as an estimation of the population at risk. A total of 11 701 cases were identified to species level. Thirteen primary clusters ($p<0.05$) were identified, of which four were long term clusters lasting more than 8 years and seven were short term clusters lasting less than 2 years. Domestic dogs accounted for 60% of all rabies cases. Wildlife was responsible for 15.8% of cases, with yellow mongoose the most frequently affected species, followed by bat-eared fox (*Otocyon megalotis*), black-backed jackal, meerkat (*Suricata suricatta*) and aardwolf (*Proteles cristatus*). Wildlife species affected by rabies followed different spatial distributions. Bat-eared fox and aardwolf predominated in western South Africa and yellow mongoose in central South Africa, and jackals mostly predominated in north-western South Africa, in both the Gauteng and North West provinces.

To investigate the feasibility of implementing oral rabies vaccination in jackals in Gauteng province, factors associated with the uptake of oral bait by free-ranging jackal and other wildlife species were evaluated in a multi-site field study. Three different baits were offered: commercial fishmeal polymer, pieces of red meat and chicken heads. Bait uptake was observed using camera traps and patterns of uptake assessed by multiple correspondence analysis and Cox proportional hazards models. In general, all baits were well accepted with an uptake of 91% by all species, and 20% of baits were taken by jackals; median consumption time of bait for jackal was 18 hours. In species other than jackals, there was a faster uptake in the winter months when less food was available and vegetation was sparse, whereas jackal showed no seasonal preference. Chicken heads may be the preferred bait type for oral vaccination of black-backed jackal in this area, and consideration should be given to placing bait during summer and at dusk, in order to minimize uptake by non-target species such as warthogs, which are only active during the day.

Two trials were conducted to assess the antibody response to oral rabies vaccine (Raboral VR-G®, Boehringer Ingelheim) in both captive and free-ranging jackals. Captive jackals (n=12) had adequate antibody titres at 4 weeks and 12 weeks and maintained sufficient antibody titres for up to 12 months (median 3.5 IU/ml; IQR: 1.5-8.25) after single oral vaccination. For testing the vaccines in free-ranging jackal habitat, four sites were baited with Raboral VR-G® vaccine: three in the northern part of South Africa and one on the central plateau, all during winter. Bait distribution, from a vehicle and on foot at these sites resulted in between 36 % and 71% of jackals tested having antibody tires to rabies after a single baiting season.

Oral bait rabies vaccination could be a useful component of a control strategy for rabies in wildlife and domestic dogs in South Africa, and further work should investigate oral bait vaccine over larger areas and multiple applications.

Key Terms

Epidemic, jackal, oral bait vaccine, rabies, South Africa, spatiotemporal analysis

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List of abbreviations

CAV2-RG	recombinant canine adenovirus (serotype 2) rabies glycoprotein
CEO	chicken embryo
CNS	central nervous system
DALLRD	Department of Agriculture, Land Reform and Rural Development
EC	Eastern Cape province
ERA	porcine kidney cells
FAO	Food and Agricultural Association of the United Nation
FS	Free State province
GARC	Global Alliance for Rabies Control
GGNP	Golden Gate National Park
GLW	gridded livestock of the world
GP	Gauteng province
ha	hectare
IQR	interquartile range
KZN	KwaZulu-Natal province
LLR	log-likelihood ratio
LP	Limpopo province
MLV	modified-live
MP	Mpumalanga province
NC	Northern Cape province
NICD	National Institute for Communicable Disease
NW	North West province
OIE	World Organization for Animal Health
ORV	oral rabies vaccine
OVI	Onderstepoort Veterinary Institute
PEP	post-exposure prophylaxis
RABV	*Rabies lyssavirus*
RNA	ribonucleic acid
RSA	Republic of South Africa
SAD	Street Alabama Dufferin
SADC	Southern African Development Community
SAG2	SAD Avirulent Gif
UAR	Unite Against Rabies campaign

VR-G®	recombinant vaccina virus-rabies glycoprotein
WC	Western Cape province
WHO	World Health Organization

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

Rabies is a viral disease caused by *Rabies lyssavirus* (RABV) belonging to the order Mononegavirales, family Rhabdoviridae, genus *Lyssavirus* (Amarasinghe et al., 2017). It is a negative-stranded ribonucleic acid (RNA) virus resulting in fatal encephalitis in all warm blooded animals, including humans (Swanepoel et al., 1993). Rabies remains a neglected tropical disease, especially of low income developing countries, in Africa and Asia (Lembo et al., 2010). It is endemic to most of Africa, with economic consequences due to human fatalities and loss of livestock (Hampson et al., 2015). Due to this, in 2018, the Unite Against Rabies campaign (UAR) was initiated by the World Health Organization (WHO), Food and Agricultural Association of the United Nations (FAO), World Organization for Animal Health (OIE) and Global Alliance for Rabies Control to eliminate human fatalities from dog-mediated rabies by 2030 (United Against Rabies Collaboration, 2019).

The domestic dog (*Canis lupus familiaris*) is still the primary vector in the transmission of rabies, with >90% of rabies cases in humans caused by dog bites (Morters et al., 2013; Sabeta et al., 2015). Rabies in dogs is endemic to southern Africa, with a higher prevalence in the densely populated rural areas of northern and eastern South Africa, (Zulu et al., 2009).

Wildlife rabies occurs in a number of species in South Africa with the yellow mongoose (*Cynictis penicillata*), bat-eared fox (*Otocyon megalotis*), and black-backed jackal (*Canis mesomelas*) being the primary vectors (Swanepoel et al., 1993). Rabies in mongoose was present in South Africa prior to the introduction of rabies by canids in the late 1800's (Van Zyl et al., 2010). The first reported case of rabies in black-backed jackal was recorded in 1947, and in bat-eared foxes in 1955 (Thomson and Meredith, 1993). Rabies epidemiology in wildlife differs between areas due to differences in host ecology. Jackals are maintenance hosts for rabies under enabling environmental conditions and densities (Bingham, Foggin, Wandeler, & Hill, 1999; Foggin, 1985). Jackal densities of 1 to 1.4 jackal/km^2 have been found to be sufficient for the maintenance of rabies within a population without external introduction (Rhodes et al., 1998). In 2011 in north-eastern South Africa, a rabies outbreak that was specific to jackal occurred, strongly suggesting that black-backed jackals in the northern parts of South Africa are capable of maintaining rabies infection independently from dogs (Zulu et al., 2009).

Introduction of rabies into the habitats of several endangered carnivores such as the wild dog (*Lycaon pictus*) and the Ethiopian wolf (*Canis simensis)* has resulted in catastropic population declines. Rabies has been implicated in the loss of 75 % of the Ethiopian wolf population in the early 1990's and continues to be a threat to the survival of the species (Randall et al., 2004). Wild dog populations have declined in both South Africa and Kenya in response to exposure to rabid domestic dogs and black-backed jackals (Alexander et al., 1995; Hofmeyr et al., 2004, 2000).

Jackals are becoming increasingly important as wildlife vectors for rabies transmission in southern Africa (Swanepoel et al., 1993; Zulu et al., 2009). Jackals are heavily persecuted in South Africa due to their predation on livestock and wildlife, as well as peoples' fear of contracting rabies. Culling strategies have been used to control rabies in yellow mongoose and black-backed jackals, but are usually only effective in the short term as it creates a vacuum which results in movement of more animals from adjacent areas, as well as disrupting territories and social structures (Cross et al., 2007).

Oral vaccination strategies have been used to control rabies in wildlife, especially in Europe and North America. Various oral vaccines administered as bait have been used over time for the successful control of rabies in meso-carnivores such as feral dogs, foxes (*Vulpes* spp.), raccoon (*Procyon lotor*) and coyotes (*Canis latrans*) in Europe and North America (Cross et al., 2007; Steelman et al., 2000; World Health Organisation, 2007). Only two oral rabies vaccines have met the minimum World Health Organization (WHO) requirements with regard to oral efficacy and safety: the attenuated SAG-2 vaccine (Virbac, France) and recombinant vaccinia virus-rabies glycoprotein (V-RG®) vaccine

(Merial, USA, now part of Boehringer Ingelheim) (World Health Organisation, 2007). The effectiveness of any oral bait vaccine depends on two factors: the uptake of the bait by the target species and the efficacy of the vaccine in the target species (Knobel et al., 2002). The WHO has defined the efficacy of a vaccine as the protection of a vaccinated animal against disease (World Health Organisation, 2007).

In other studies that trialled bait items for preference fish meal polymer was well accepted (100%) by golden jackal (*Canis aureus*) in Israel (Linhart et al., 1997), chicken heads were the preferred bait in the African wild dog (*Lycaon pictus*) (Knobel et al., 2002) and goat meat pieces for Ethiopian wolves (*Canis simensis*) (Sillero-Zubiri et al., 2016). However, bait uptake depends not only on the bait type, but also time of baiting, mode of bait distribution and bait density and variation in update by target species throughout the year (Be-Nazir et al., 2018). These are important variables to be included in any studies of bait preference.

Only once bait preference has been determined can oral vaccine strategies for wildlife be implemented. This has been highly effective in Europe, where the red fox *(Vulpes vulpes*) is the main wildlife reservoir, and has resulted in the eradication of rabies from Austria, Belgium, Czech Republic, Finland, France, Germany, Italy, Luxembourg, Netherlands and Switzerland in Europe (Müller et al., 2015). However, this took a total of ten different rabies vaccines in multiple campaigns over a 30-year period (Müller et al., 2015). A campaign was defined as a continuous vaccination program using a single vaccine type (Müller et al., 2015).

A V-RG® vaccine was initially developed as a safer vaccine than the attenuated vaccines for red foxes in Belgium (Maki et al., 2017). It has since been used successfully on a variety of wild carnivores such as red foxes in Europe, striped skunks (*Mephitis mephitis*), raccoons (*Procyon lotor*), artic foxes (*Vulpes lagopus*) and grey foxes (*Urocyon cinereoargenteus*) in the USA, and red foxes and golden jackals in Israel (Maki et al., 2017; Müller et al., 2015; Yakobson et al., 2006). The red fox in Europe occupies a similar ecological niche to jackals in southern Africa (Rhodes et al., 1998). The advantage of the V-RG® vaccine is its safety, with no risk of reverting to virulence in non-target species, including humans. A further advantage of the V-RG® vaccine is its stability in the environment with up to three weeks stability at an environmental temperature between 8 and 37°C (Maki et al., 2017), compared to the SAG-2 vaccine, which deteriorated in temperatures above 30°C (Mähl et al., 2014). This makes the V-RG® vaccine a suitable candidate to test in the South African context.

The V-RG® vaccine's efficiency and effectiveness has not been established in any meso-carnivores in South Africa. Rabies antibody titres above 0.5 IU/ml are considered to be adequate for protection against rabies in dogs, while titres above 0.25 IU/ml are considered adequate in foxes (*Vulpes* spp), raccoons and raccoon dogs (*Nytereutes procyonoides*) (Moore et al., 2017; Office International Des Epizooties, 2008). For black-backed jackal and side-striped jackal (*C. adustus*) titres of 0.5 IU/ml are adequate for protection against field strains of rabies (Bingham et al., 1999b). There is no single cut-off level of rabies antibody titre that is invariable protective as small number of animals succumb to infection despite adequate antibody levels and some animals with no antibody titres survive infection (Moore et al., 2017; Moore and Hanlon, 2010).

In South Africa dog rabies is currently controlled by mass parental vaccination both by private and state veterinarians (LeRoux et al., 2018). Oral vaccination has been used in dogs, which were difficult to handle (Cliquet et al., 2018). In wildlife vaccination of the endangered African wild dog against rabies has been done both in captive (Knobel et al., 2003; van Heerden et al., 2002) and free-ranging setting, both with parental vaccine and oral rabies vaccine (SAG-2) and has resulted in a reduction of rabies cases in the species (Hofmeyr et al., 2004).

Oral rabies vaccination has to date not be done in other wildlife in South Africa for control of rabies in reservoir hosts. Thus, for control of rabies in wildlife in South Africa there are still knowledge gaps that need to be addressed.

1.2 General research aims

The aim of this thesis is to significantly contribute to the knowledge base of our understanding of rabies distribution in South Africa, to what extent wildlife vectors play a role in transmission and maintenance of endemic rabies, and of solutions to reduce rabies especially in jackals.

The aim is to provide a complete and current overview of the spatiotemporal patterns of rabies vectors in South Africa to allow conservation biologists, protected area managers and government to focus rabies control strategies in areas where significant and long-term clusters in rabies cases are present.

This thesis focusses specifically on wildlife species, using the black-backed jackal as a case study for investigating suitable rabies control measures. These data provide a strong scientific basis for i) enhancing the understanding of wildlife in the epidemiology of rabies, and ii) guiding in the best control strategies for control of outbreaks in wildlife. In order to achieve this, the following research questions were answered:

What is the recent spatiotemporal distribution of rabies in South Africa?
A spatiotemporal analysis of rabies in South Africa from 1993 to 2019 was performed to determine temporal and spatial clusters in domestic animals and wildlife, as spatial data became more reliable after the establishment of the current municipalities in South Africa.

What was the involvement of wildlife in the significant clusters identified?
A review of involvement of wildlife species in rabies clusters was performed and cases 6 months prior to the start of the individual clusters were also reviewed.

What is the best method for baiting black-backed jackal for oral rabies vaccination?
A bait preference study was performed evaluating the response of jackal, other carnivores and non-target species to three different bait types in a free-ranging situation, assessing bait preference per species, as well as time and season for patterns of preference.

What is the seroconversion proportion and rabies antibody titre in response to oral rabies vaccination in a controlled environment?

Titres and duration of titres in 12 captive jackals were evaluated for up to 48 weeks (12 months) after vaccination.

What is the prevalence of seroconversion in free-ranging jackals before and after oral rabies bait placement?

Free-ranging jackals were captured, and rabies antibody titres were evaluated prior to and up to 92 weeks (23 months) after oral rabies vaccine in two areas in South Africa.

1.3 Organisation of the thesis

Chapter 1 provides a general overview of the thesis

Chapter 2 reviews the current literature on rabies, with a focus on southern Africa and the behaviour of black-backed jackals in the context of rabies epidemiology.

Chapter 3 reviews and analyses the spatiotemporal patterns of rabies in South Africa over the last 26 years (1993 to 2019) and evaluates the most significant clusters that occurred during that time period. It also evaluates the temporal and seasonal patterns of rabies in wildlife and domestic animals.

Chapter 4 evaluates the oral bait preference of free-ranging black-backed jackal in response to three different bait types (commercial fishmeal polymer, red meat and chicken heads) and proposes the best bait and placement of bait for targeting jackals.

Chapter 5 evaluates the efficacy of oral recombinant vaccinia virus rabies vaccine in captive black-backed jackals over a 12-month period. The sero-prevalence of rabies antibody titres in free-ranging jackals prior to and after oral rabies bait placement in two areas in South Africa was then evaluated.

Chapter 6 concludes with an integrative synthesis of major findings, within the context of literature and current knowledge. Recommendations are made for the vaccination of black-backed jackal to reduce rabies cases in wildlife and domestic animals in South Africa.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview

Rabies is a zoonotic viral disease that causes encephalomyelitis in all warm-blooded animals, including humans, caused by a single-stranded, negative sense, non-segmented RNA virus (Nel and Markotter, 2007). It is responsible for at least 59 000 human deaths annually (Hampson et al., 2015). The majority of human deaths occur in Africa and Asia, with India having both the highest levels of human exposure and human deaths due to rabies. The second highest death rates due to rabies occur in the Congo basin and Southern African Development Community (SADC) regions (Hampson et al., 2015). These figures are thought to be an underrepresentation of human rabies deaths in the developing world, with only an estimated 3% of human deaths due to rabies being reported (Knobel et al., 2005). Rabid domestic dogs (*Canis lupus familiaris*) are responsible for over 90% of the cases of human exposure (Franka et al., 2013; Kaare et al., 2009). The economic burden of rabies is not only due to the loss of human life and livestock but also due to the high cost of post-exposure treatment, which has been estimated at $40 per human case in Africa, including travel to the medical facility (Lembo et al., 2010). In 2018 the Unite Against Rabies campaign (UAR) was initiated by the World Health Organization (WHO), Food and Agricultural Association of the United Nations (FAO), World Organization for Animal Health (OIE) and Global Alliance for Rabies Control to eliminate human death from dog mediated rabies by 2030 (United Against Rabies Collaboration, 2019). This goal is focusing on the reduction of human death with 83% of human cases associated with domestic dogs in South Africa. Wildlife is directly responsible for only a small number of human cases but wildlife is often linked to outbreaks in domestic dogs and livestock, which in return result in human exposures. Reduction of rabies in wildlife specifically black-backed jackal would also reduce the risk of rabies in other wildlife. The spread of infection from dogs to other canids is common, and has detrimental impacts on endangered canids such as the African wild dog (*Lycaon* *pictus*) and Ethiopian wolf (*Canis simensis*), with secondary knock-on effects such as losses in ecotourism (Alexander et al., 1993; Laurenson et al., 1997b; Randall et al., 2004).

Effective dog vaccination strategies can eliminate rabies from countries if there are no wildlife reservoir host. In Central and South America, massive, synchronised dog vaccination campaigns have been successful in reducing the number of rabies cases in both dogs and humans (Belotto et al., 2005). The most successful rabies control programs across the world are those which have taken a multiagency approach (Brochier et al., 1996). In North America this approach to the management of rabies in wildlife has been the most effective (Rosatte et al., 2007). The use of oral bait vaccine has been more effective than culling, especially in raccoons (*Procyon lotor*) (Rosatte and Lawson, 2001). The failure of rabies control in Africa is thought to be related to the low priority being given to rabies control, low vaccination coverage, presence of wildlife vectors, lack of accessibility to dog populations and limited resources (Lembo et al., 2010).

2.2 Aetiology

Rabies is caused by *Lyssavirus* belongs to the Rhabdoviridae family of the *Mononegavirales* order. The virus most likely developed in bats and then adapted to canine hosts approximately 500 to 1400 years ago (Badrane and Tordo, 2001; Holmes et al., 2002). The rabies-like symptoms described in antiquity were most likely caused by other lyssaviruses with the current lyssavirus having developed around 10 000 years ago (Badrane and Tordo, 2001) although this is difficult to confirm due to lack of virus available for genetic analysis.

The *Lyssavirus* genus has been subdivided into three phylogroups, based on genetic distance and serological cross-reactivity (Be-Nazir et al., 2018). Phylogroup I contains *Rabies lyssavirus* (RABV), *European bat 1 lyssavirus*, *European bat-2 lyssavirus*, *Bokeloh bat lyssavirus*, *Duvenhage lyssavirus*, *Australian bat lyssavirus*, *Arvan lyssavirus*, *Khujand lyssavirus* and *Irkut lyssavirus*. Phylogroup II contains *Lagos bat lyssavirus*, *Mokola lyssavirus* and *Shimoni bat lyssavirus*. Phylogroup III contains *West Caucasian bat lyssavirus* and *Ikoma lyssavirus*. RABV can phylogenetically be divided into Africa 1, Asia, Arctic rabies, European-Middle East, and Latin America 1 and 2. European and African strains are closely related (Bourhy et al., 1993) (Figure 2.1).

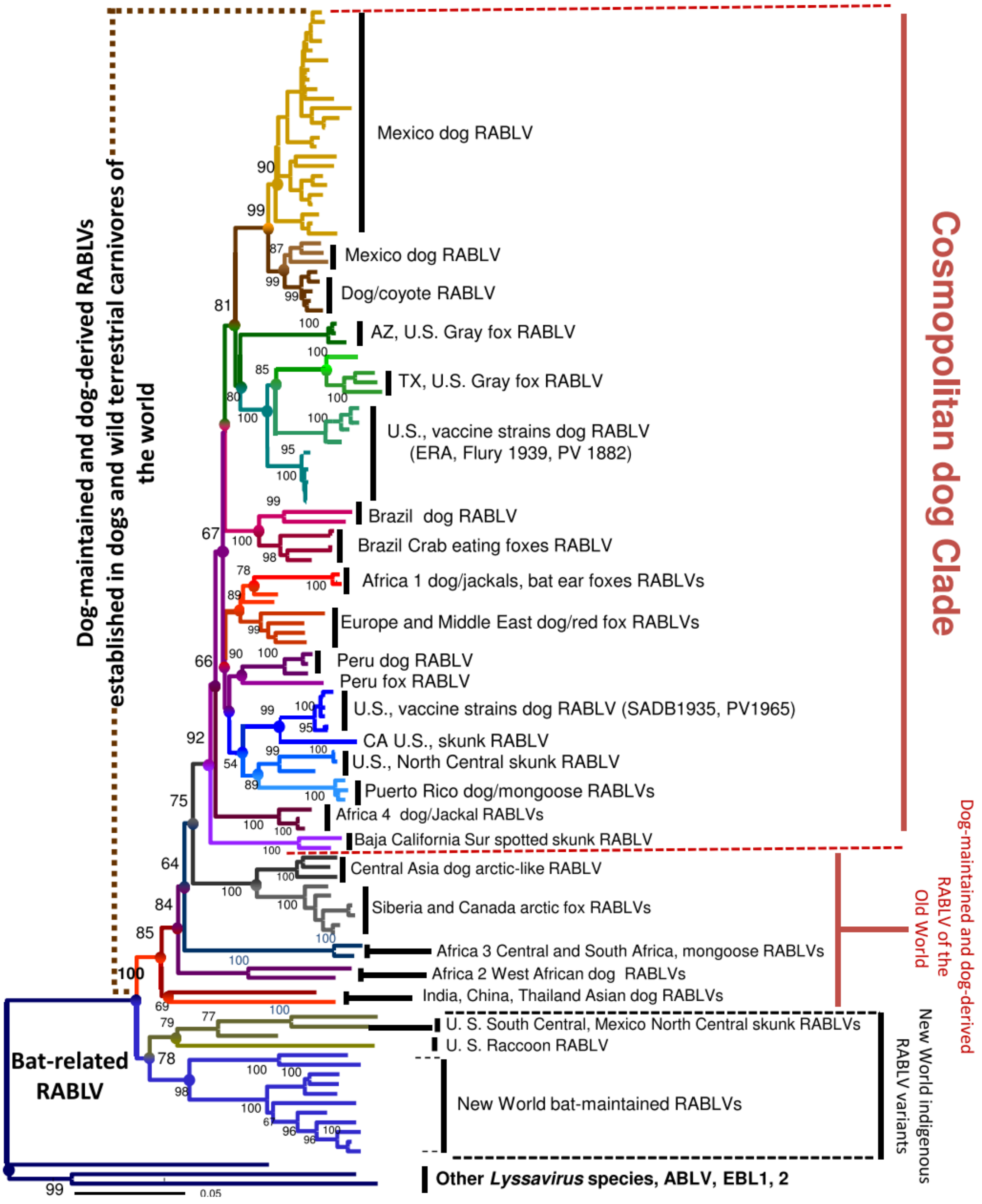

Figure 2.1 Phylogeny of *Rabies lyssavirus* circulating in dogs, mesocarnivores and bats in the New World (Velasco-Villa et al., 2017b)

RABV is found in all warm blooded animals and bats in the New World, while the reservoir host of other lyssaviruses are bat species found in the Old World with the exception of *Mokola lyssavirus* and *Ikoma lyssavirus* in which the reservoir species has not been identified to date. The *lyssavirus* has been classified in a small proportion of all recognized bat species, but only vampire bats are considered significant as a public health risk associated with rabies infection (Banyard et al., 2013). The common vampire bats (*Desmodus rotundus*) became the main reservoir for infection in Central and South America in the 1990s (Ulloa-Stanojlovic and Dias, 2020). There has been an increase of cases in cattle due to vampire bats, which are consequently considered a significant health risk both to cattle and humans (Ulloa-Stanojlovic and Dias, 2020).

The rabies genome (Figure 2.2) contains 12 kilobases of RNA, which encodes five viral proteins (nucleoprotein [N], phosphoprotein [P], matrix protein [M], glycoprotein [G] and RNA-dependant RNA polymerase [L]) (Nel and Markotter, 2007). The lyssavirus particle is bullet-shaped 100-300 nm long and 75 nm in diameter. It comprises two structural and functional units: the internal helical nucleocapsid and the external envelope (Be-Nazir et al., 2018). Knobbed glycoprotein protein spikes bind the virions to the host cell receptors (Graham et al., 2008).

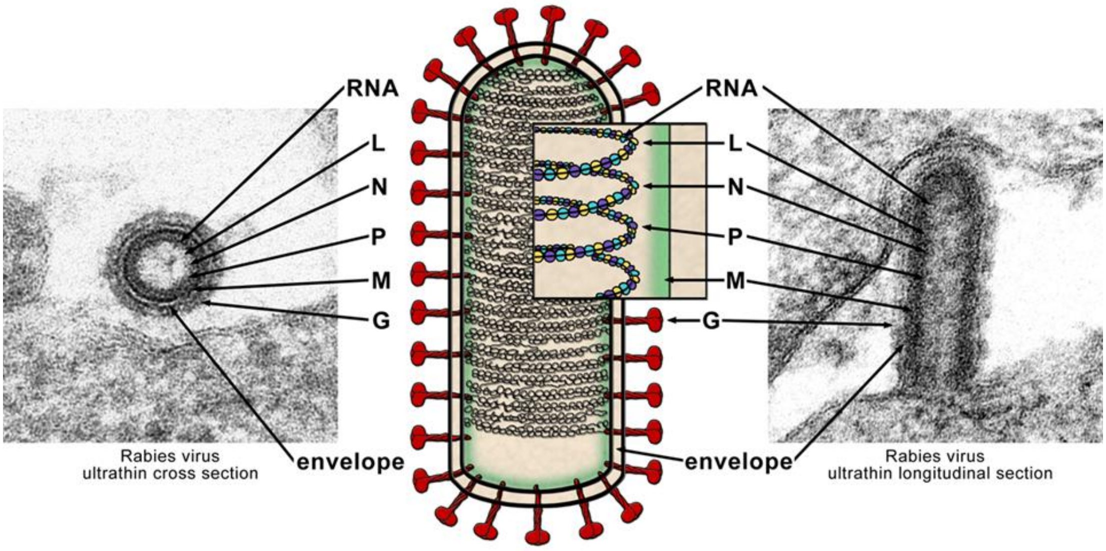

Figure 2.2: Rabies virus showing the five viral proteins (nucleoprotein [N], phosphoprotein [P], matrix protein [M], glycoprotein [G] and RNA-dependant RNA polymerase [L]) and cross-sectional structures. (World Health Organisation (WHO), 2020)

2.3 Pathogenesis

The pathogenesis of RABV depends upon the portal of entry, the host species, the pathogenicity of the virus, and the dose of virus. The majority of infections from insectivorous bats occur through superficial bites and the virus enters the nervous system through the sensory nerve endings in the skin (Begeman et al., 2018). This is in contrast to carnivore bites were infection enters through motor nerve endings in the skeletal muscle (Begeman et al., 2018). The virus can replicate in muscle or localised tissue before gaining access to motor endplates and axons. Virions are carried to the central nervous system in a transport vesicle by fast retrograde transport along motor axons, with no propagation in sensory, sympathetic or parasympathetic pathways (Ugolini, 2011). At this time the virus is no longer detectable at the site of the portal of entry (Swanepoel, 2004). The incubation period for RABV is determined by the site of entry e.g. in the head or forelimb results in shorter incubation periods as closer to the brain (Soulebot et al., 1982).

Once the virus has entered the nervous system it is transported by retrograde axoplasmic flow to the central nervous system (CNS). Replication can also occur in the muscle, which results in intermediate incubation periods (Baer and Cleary, 1972). The incubation period also depends on the strain of the virus and infective dose (Fekadu et al., 1982). Motor neurons become infective within 18 hours after intramuscular inoculation, and by day three there is a massive invasion of the spinal cord and sensory ganglia in mice (Coulon et al., 1989). Once the virus has entered the brain, the spread of infection is rapid (Swanepoel, 2004). From the CNS the virus moves via slow anterograde axoplasmic flow in motor neurons to the ventral roots and nerves (Ugolini, 2011). The virus has a predilection for the limbic system, which results in the characteristic 'rabid' clinical signs, including loss of fear. RABV can be found in the salivary gland before the onset of clinical signs (Fekadu et al., 1982).

Domestic cats (*Felis catus*) have shown to be more resistant to infection when compared to dogs and sheep (Soulebot et al., 1982). Mice and other laboratory species, skunks and the greater kudu (*Tragelaphus strepsiceros*) are susceptible to oral infections. Kudus often have lesions in the oral mucosa due to browsing on thorny *Vachellia* species, which increases their risk of infection (Hübschle, 1988).

2.4 Clinical Signs

Clinical signs vary between species. In humans, the mean incubation period was 6.9 weeks (range 2 to 17 weeks) in Zimbabwe (Foggin, 1988). The most common clinical signs in human cases are excitation and mental disturbance, followed by hypersalivation and visual hallucination (Foggin, 1988; Salomão et al., 2017). Less than half of the cases showed vomiting, convulsion, photophobia, headaches or fever (Salomão et al., 2017). In humans, death usually occurs within 7 to 10 days after the appearance of clinical signs and the furious form is usually associated with higher viral load and lower immune response (Hemachudha et al., 2013).

The incubation period in dogs is variable, but usually between 2 to 8 weeks. A mean incubation period of 3.6 weeks was seen in Zimbabwe (Foggin, 1988), which is slightly shorter than the 3.9 weeks reported in Tokyo during the 1950s (Tojinbara et al., 2016). A febrile phase with subtle changes in temperament is seen initially, followed by the acute neurological or furious form, with the infected dog becoming increasingly irritable and aggressive. This is followed by paralysis of the laryngeal muscles and may lead to development of seizures and death (Swanepoel, 2004). The majority of dogs present with a furious form (76%), while 8% of dogs develop a so-called dumb-form, with dropping of the jaw and paralysis, and the remainder have a mixture of clinical signs (Foggin, 1988). Cats, however, tend to develop the furious form when infected (Swanepoel, 2004).

An incubation period of 2 to 4 weeks is seen in sheep and goats, shorter than in cattle where a mean incubation of 5.3 weeks is seen (range 2-12 weeks) (Foggin, 1988). Half of infected cattle show aggressive behaviour, while others are seen standing away from the herd, anorexic and docile (Brückner et al., 1978). Clinical signs in small stock are similar to those in cattle (Swanepoel, 2004). In horses, an incubation period of 2-9 weeks has been noted, with clinical signs including behavioural changes, especially aggression, colic and biting at the site of infected wounds (Swanepoel, 2004; Turner, 1994).

In side-striped and black-backed jackals, 56% of infected individuals showed aggressive behaviour, while the rest show other changes in behaviour, such as loss of fear of humans and dogs (Foggin, 1988). The incubation period in experimentally infected side-striped and black-backed jackals varied from 10-31 days, with a mean of 16 days (Bingham et al., 1999b; Foggin, 1988).

Incubation periods in experimentally infected mongoose varied between 12-107 days with a clinical phase of 2 to 7 days. Yellow mongoose (*Cynictis penicillata*) showed behavioural changes, including loss of fear or increased shyness, with vocalisation being common (Chaparro and Esterhuysen, 1993).

Behavioural changes in greater kudu include loss of fear and lack of awareness to their surroundings and kudu are often found wandering into houses. Salivation is also a common finding, followed by ataxia, knuckling over at the fetlock and incoordination, followed by death (Hübschle, 1988).

2.5 History of RABV around the world: vectors

This thesis focusses on RABV. The rabies virus can form a stable infective cycle with transmission via infected saliva in the host, as mostly seen in carnivores such as the dog, red fox, black-backed jackal, side-striped jackal and golden jackals, raccoon and a variety of bat species. The virus has the ability for cross-species transmission resulting in infection in humans with no further transmission (Begeman et al., 2018). Occasionally the virus can adapt to a new host and result in the transmission of the virus within that species (Holmes et al., 2002). The factors that allow the virus to become established in a new species are ecological, genetic, and behavioural factors of the host species (Holmes et al., 2002).

2.5.1 Europe: dogs, grey wolves, red fox, racoon dog

Rabies is an ancient disease in Europe and the Mediterranean basin. Before the 20th century it was found mainly in dogs and grey wolves (*Canis lupus*) (Nel and Markotter, 2007). With effective vaccination campaigns, the presence of rabies in dogs has dramatically declined. Britain eradicated rabies in dogs in 1922 (Holmala and Kauhala, 2006). It was well controlled in dogs by 1970 in the rest of Europe, but rabies continued in the red fox population. In Europe, 57% of reported rabies cases occurred in dogs and 33% in wildlife species in 1997 (Holmala and Kauhala, 2006).

As the rabies virus spread west and south in Europe, two host species changes occurred: the first from the dog to the red fox, and the second from the red fox to the raccoon dog (*Nyctereutes procyonoides*) (Bourhy et al., 1999). The red fox became the main vector in Europe by 1945 (Holmala and Kauhala, 2006). Red foxes have a complex and variable social structure (Macdonald and Bacon, 1982). Territory sizes vary depending on food and den availability. Home ranges in urban areas are generally smaller by three-fold as opposed to rural areas (Macdonald and Bacon, 1982). Fox home ranges vary from 50 to 1 500 ha, and the threshold density for rabies spread is estimated at 0.63 individuals/km^2 in central Europe (Holmala and Kauhala, 2006).

Fox densities in eastern and northern Europe are usually below the threshold for maintenance of rabies in the population. The density of foxes required is influenced by the environment, with a density of 0.3 foxes/km^2 being sufficient for rabies spread in the arid steppes of western Siberia, where home ranges of foxes are much larger (Tyul'ko and Kuzmin, 2002).

The raccoon dog has become an important vector of rabies in eastern Europe, especially the Baltic States (Holmala and Kauhala, 2006). It is an invasive species from East Asia, which was imported into western Russia in the first half of the 20th century. Raccoon dogs hibernate from November to March but might change den sites during the winter period (Singer et al., 2009). Hibernation might prolong the rabies incubation period, complicating the disease transmission cycle (Singer et al., 2009). Rabies epidemics in raccoon dogs depend on the density of raccoon dogs in an area, hibernation status and the presence of other vectors (Singer et al., 2009). Home ranges of raccoon dogs are between 150-700 ha (Holmala and Kauhala, 2006). Densities of foxes and raccoon dogs can combine to reach densities sufficient to maintain rabies in a population (Holmala and Kauhala, 2006).

2.5.2 Arctic: dogs and Arctic fox

Rabies is endemic in the Arctic. It can be traced back to the 1600's, most likely associated with European colonisation (Velasco-Villa et al., 2017b). The first human case was reported in 1819 (Tabel et al., 1974). Rabies has been reported in both the Arctic fox (*Vulpes lagopus*) and dogs (Mørk et al., 2011). The isolate is specific to the Arctic and distinct from the isolate found in red foxes. Clinical signs in Arctic foxes are reported to be very short, only lasting 1-2 days with a prolonged incubation period of up to 6 months. Rabies is spread over long distances by Arctic foxes travelling over the sea ice (Mørk et al., 2011). In Alaska, cycles of rabies in Arctic foxes occur every 3-4 years with a peak in February (Ballard et al., 2001; Mørk et al., 2011).

2.5.3 Middle East : dog, golden jackal, red fox

Rabies was present in the Middle East before the increase in urbanisation in the 1800s. The first record of rabies is from 4000 years ago in Mesopotamia, in modern-day Iraq (Horton et al., 2015). The current rabies strains are thought to be from dog introductions via Europe (Horton et al., 2015). Wildlife does not present independent cycles from the dog in most of the Middle East, except for Israel where the golden jackal and red fox are important in rabies transmission (Yakobson et al., 2006).

2.5.4 Asia: dog, Arctic fox, raccoon dog and ferret badger

Rabies in China was introduced by the Arctic fox with an increase in rabies cases since the 1950 (Shao et al., 2011; Zhang et al., 2005). Rabies was introduced into the Philippines around the 1900s by dogs (Chang et al., 2015). While the raccoon dog is the primary vector of rabies in northern China, the ferret badger (*Melogale moschata*) is the primary vector in southern China and Taiwan. Taiwan was free of canid rabies from 1961 until 2010, when it was detected in ferret badgers (Chang et al., 2015). In India the domestic dog is the main vector for rabies, with a third of human death worldwide occurring in India (Fitzpatrick et al., 2016).

2.5.5 America: skunk, dog, red fox, coyote, grey fox, Indian mongoose, crab-eating fox, marmoset, kinkajou and coati

Rabies is thought to have arrived in the Americas with the arrival of European settlers. It is thought that it become established in the 1700s with the increase in dog numbers with the first rabies epizootic in dogs in Mexico City confirmed in 1709 (Velasco-Villa et al., 2017b). RABV then spread from dogs to several meso-carnivores (Velasco-Villa et al., 2017a). The first epizootic in wildlife was seen in spotted skunks (*Spilogale putorius*) in 1826 (Velasco-Villa et al., 2017b).

Wildlife now accounts for more than 90% of cases in North America to date (Ma et al., 2020). With the change in agricultural practices and land-use, wildlife rabies increased (Smith et al., 1995). The highest reported cases of rabies in wildlife are in the agricultural lands of southern Ontario, where the main wildlife host is the red fox, followed by the striped skunk (*Mephitis mephitis*) (MacInnes, 1988). The strain found in the red fox originated from Arctic foxes in the 1950s. Red foxes are responsible for RABV in the north- eastern parts of the USA (Smith et al., 1995). Rabies is found in the Appalachian mountains and Arizona in grey foxes (*Uracyon cinereoargenteus*) (MacInnes, 1988). Skunks are associated with rabies in the Mississippi River valley, Minnesota, Iowa and Texas (MacInnes, 1988). Raccoons are associated with rabies in Florida, spreading to Virginia, Maryland and Pennsylvania (Figure 2.3). Three distinct clades of raccoon rabies have been recognised (Smith et al., 1995). Isolates from raccoons show great sequence homology and outbreaks are associated with being close to human settlements (Smith et al., 1995). Up until 2013 raccoons were the most important wildlife reservoir for rabies, followed by skunks, bats and foxes (Rosatte, 2013). Recently the incidence of rabies in the coyote (*Canis latrans*) has increased (Slate et al., 2009, 2005). The first case of rabies in a coyote was recorded in 1988 in Texas, with the frequent transmission of rabies between dogs and coyotes (Smith et al., 1995).

The small Indian Mongoose (*Herpestes auropunctatus*) was introduced into the Caribbean islands in the early 1900s to control rats (Berentsen et al., 2015; Linhart et al., 1993). It was responsible for rabies in Grenada and other Caribbean islands, with the occasional spill over into the dog (Berentsen et al., 2015), while the dog is also the main reservoir in

Haiti and the Dominican Republic (Seetahal et al., 2018). Vampire bats are the main host in Trinidad, Guyana, Suriname and Guyana (Seetahal et al., 2018).

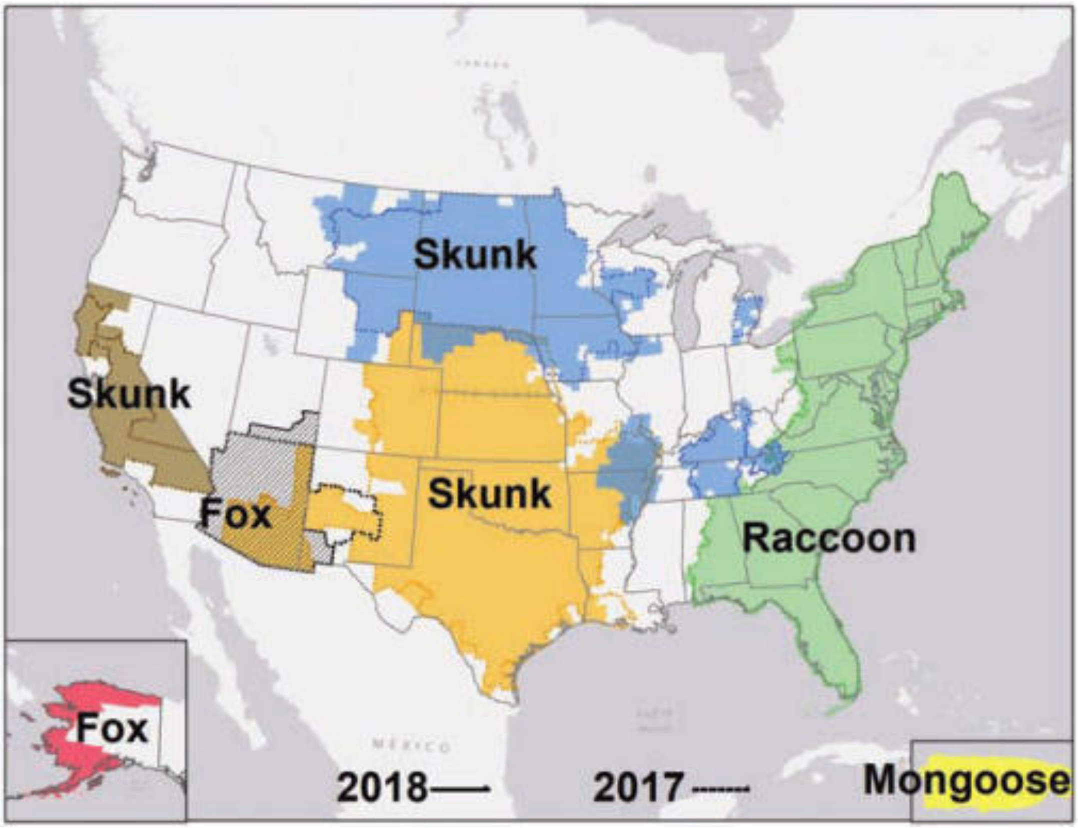

Figure 2.3: Distribution of rabies virus in mesocarnivores in the USA and Puerto Rico from 2013 to 2018 (Ma et al., 2020).

The marmoset (*Callithrix jacchus*), crab-eating fox (*Cerocyon thous*), kinkajou (*Potus flavus*) and the coati (*Coati coati*) have been associated with rabies in South America (Favoretto et al., 2013, 2001). Rabies in the crab-eating fox most likely originates from the dog (Favoretto et al., 2013), while rabies in the coati was associated with rabies isolates found in bats, and not RABV (Aréchiga-Ceballos et al., 2010).

2.6 Rabies in Africa

Rabies has been present in northern Africa since antiquity but is now associated with urban areas due to greater population of dogs (Swanepoel, 2004). The first cases were reported in West and East Africa at the beginning of the 19th century (Swanepoel, 2004).

An estimated 21 476 human deaths occur each year in Africa (Hampson et al., 2015). However, the focus of this thesis is southern Africa, including Southern African Development Community (SADC) member states neighbouring South Africa. The first reported case of rabies in South Africa was in the historical Cape province in 1882, from an infected dog that was imported from England (Swanepoel et al., 1993). Rabies was then reported in Namibia in 1885. There was an outbreak in dogs in 1902 in southwestern Zimbabwe. Rabies was first diagnosed in dogs in Zambia in 1913 and shortly after in Botswana in 1922 (Swanepoel et al., 1993). The dog is the most important host of rabies in Zambia , associated with 69% of cases (Munang'andu et al., 2011). The incidence of rabies has been decreasing in Zambia since 1985 and is associated primarily with rural settlements (Munang'andu et al., 2011; Röttcher and Sawchuk, 1978).

In Namibia, rabies in dogs is closely linked to rabies in the black-backed jackal population (Courtin et al., 2000). Transmission cycles of RABV in dogs are mainly seen in the northern communal areas and around the urban areas of the capital Windhoek (Hikufe et al., 2019). Dogs are associated with 45% of rabies cases in Zimbabwe and appear to be unrelated to rabies cases found in wildlife (Bingham et al., 1999a).

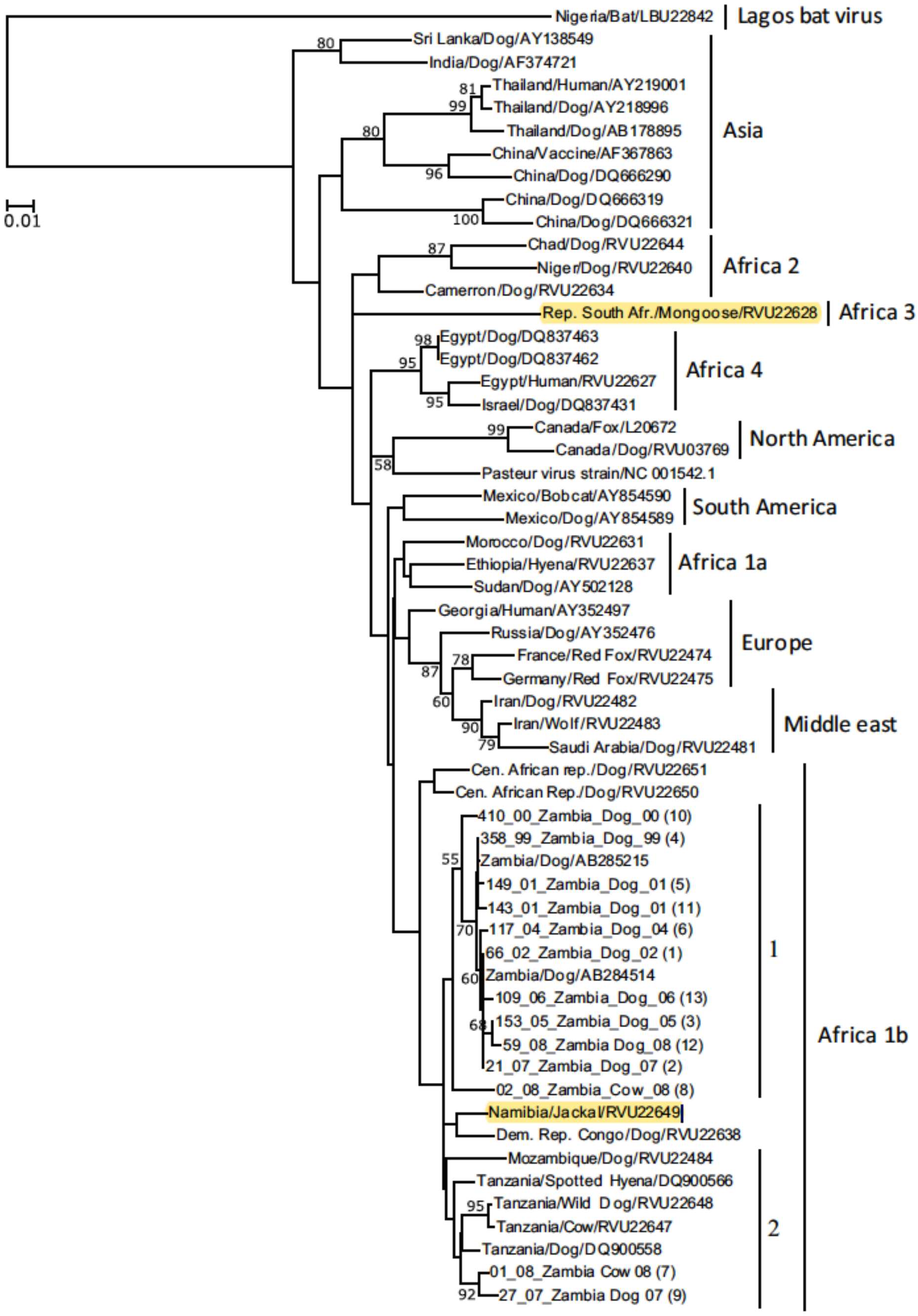

Figure 2.4: Phylogenetic analysis of rabies viruses isolated from Africa and other areas of the world based on 462nt sequence of the nucleoprotein gene. Bootstrap values for 100 replicates are indicated as percentages (<90%). Jackal strain from Namibia and Mongoose strain from South Africa are highlighted in yellow (Muleya et al., 2012).

Dogs are the most important vector in Mozambique (Dias et al., 1985), where RABV is associated with a high number of human deaths due to poor post-exposure prophylaxis (Salomão et al., 2017). Molecular evaluation of rabies viruses showed a spread of rabies between northern South Africa, Mozambique and Zambia, most likely due to movement of animals across international borders (Coetzer et al., 2017a) (Figure 2.4). Canine rabies was introduced from KwaZulu-Natal Province, South Africa into the Kingdom of Lesotho in 1982. Rabies is present throughout Lesotho, due to low canine vaccination coverage, since large parts of the country are inaccessible by road (Coetzer et al., 2017b). There is a frequent exchange of rabies via dogs between Lesotho and the Free State province, South Africa (Ngoepe et al., 2009). Despite the presence of several wild carnivore species in Lesotho, to date there have been no recorded rabies in wildlife. Rabies was introduced into Eswatini (formerly Swaziland) from Mozambique in 1954 (Swanepoel, 2004). The dog is the primary vector in Eswatini, constituting 78% of cases reported (Dlamini, 1999).

2.6.1 Wildlife canid vectors in southern Africa

There are three canids with independent maintenance capabilities in southern Africa the bat-eared fox (*Otocyon megalotis*), the black-backed jackal and the side-striped jackal (Bingham, 2005). The aardwolf (*Proteles cristatus*) and the yellow mongoose play an important role in the epidemiology of rabies. There are two endangered carnivores: the wild dog and the Ethiopian wolf, that have been severely affected by rabies outbreaks (Hofmeyr et al., 2004; Sillero-Zubiri et al., 1996; Woodroffe et al., 2012; Woodroffe and Sillero-Zubiri, 2012).

The aardwolf has recently gained importance in rabies epidemiology in South Africa. The aardwolf is found in two distinct populations; one in Egypt, Somalia, parts of Ethiopia and extending south to Tanzania, while the southern population is found in Namibia, Botswana, Mozambique and throughout South Africa with the exception of the forest region on the south coast (Skinner and Chimimba, 2005a). Rabies in aardwolf is sporadic and mainly seen in the Western and Northern Cape provinces with 53 cases reported between 1928 and 2000. (Swanepoel, 2004; Swanepoel et al., 1993). It is to date not capable of independent rabies cycles but there is a potential for the aardwolf to become a maintenance host of rabies.

Bat-eared foxes are not true foxes, but belong to a monotypic genus, *Otocyon*. There are two distinct population of bat-eared foxes in Africa. They are found in East Africa from southern Ethiopia, Somalia, Kenya and eastern Uganda to northern Tanzania. In the southern area they are found in the southern part of Namibia, Botswana and narrowly into western Zimbabwe. In South Africa they are found marginally in Limpopo province and North West provinces, and are widespread in the Northern Cape, the inland region of the Eastern Cape and coastal areas of the Western Cape (Skinner and Chimimba, 2005a). The first cases of rabies in the bat-eared fox was diagnosed in 1955/56 and it then became established in the species in South Africa (Thomson and Meredith, 1993). Despite their wide distribution, rabies in bat-eared foxes in South Africa occurs mainly in the drier western parts of the country.

The black-backed jackal is found in two separate geographical areas. The north area from the Gulf of Aden to southern Tanzania and the southern region from the south-west part of Angola to South Africa. In parts of East Africa, the black-backed jackal co-exists with the golden wolf. In southern Africa the black-backed jackal is found throughout Botswana except the extreme north-east, Zimbabwe throughout the south-west and eastwards to Mozambique north of the Save River, and in South Africa they are found throughout as well as in Eswatini and Lesotho (Skinner and Chimimba, 2005b).

The side-striped jackal is more widely distributed than the black-backed jackal in southern Africa. They are found from south of the Sahara into South Africa. They occur in the north of Namibia and Botswana and are widespread in Zimbabwe and Mozambique, their distribution reflecting the higher rainfall of these areas (Bingham et al., 1999; Skinner and Chimimba, 2005c). In South Africa they are found in the provinces of Mpumalanga, Limpopo, and into the northern part of KwaZulu-Natal and adjacent to Eswatini (Skinner and Chimimba, 2005c).

Jackals are thought to be capable of maintaining rabies independently from dogs under specific environmental conditions such as high jackal density and increased population turnover (Bingham, Foggin, Wandeler, & Hill, 1999; Foggin, 1985). Side-striped and black-backed jackal have been responsible for 25% of cases of rabies in animals in Zimbabwe since 1950 (Foggin, 1985). Rabies in jackals is most prominent in the south-east of the country. The population density of jackals is usually too low to maintain rabies independently and regular introductions from dogs results in rabies epidemics in, especially, side-striped jackals (Rhodes et al., 1998), with the majority of cases occurring in the commercial farming sectors (Bingham et al., 1999). The majority of rabies cases occur in the side-striped jackal, mainly in the northeast of the country and in black-backed jackal in the southwestern part of the country. Rabies outbreaks in jackals in Zimbabwe usually occurs every 4 to 8 years with a peak in rabies between July to October (Bingham and Foggin, 1993; Huebschle, 1999). Due to outbreak of rabies in jackal occurring after outbreaks in domestic dogs in Zimbabwe , it was suggested that jackal are not able to maintain rabies infection independently from domestic dogs. This is in contrast to Rhodes et al. (1998) findings that jackal densities of 1 to 1.4 jackal/km^2 are suitable for maintaining rabies infections without external introduction . In South Africa phylogenetic evidence suggest that black-backed jackals are able to maintain rabies infection in the north-eastern parts of the country without external introduction (Zulu et al., 2009). If jackals are capable of maintaining rabies infection in a country without introduction from domestic dogs either on their own or together with other wild carnivore communities then vaccination of dogs alone will not be sufficient to eliminate rabies (Haydon et al., 2002).

2.6.2 Conservation implications of RABV

The Ethiopian wolf is the rarest of the canid species with fewer than 500 individuals remaining in the wild. It is listed as Endangered (Marino and Sillero-Zubiri, 2011). The population is found throughout the Ethiopian highlands, with the majority found in the Bale and Simien Mountain National Parks (Claudio Sillero-Zubiri, 1992; C Sillero-Zubiri et al., 1996). The largest population is found in the Bale Mountain National Park in Ethiopia (Negi, 2014). The endangered Ethiopian wolf is commonly known as the Simien jackal, but scientifically is not listed as a jackal and is phylogenetically more closely related to the golden jackal (Gopalakrishnan et al., 2018; Rueness et al., 2011). These wolves occur in packs of up to 13 individuals, with mean pack size of seven individuals (Sillero-Zubiri and Gottelli, 1992). If disease gets introduced into a wolf pack then the entire pack is usually infected due to the their close social interactions (Gascoyne et al., 1993).

Another pack canid, the African wild dog has declined dramatically over the last 30 years and is especially threatened as populations are often not viable due to the very low densities at which wild dogs occur at (Woodroffe and Ginsberg, 1999). It is currently listed as Endangered with a decreasing population trend (Woodroffe and Sillero-Zubiri, 2020). Due to their occurrence at a natural low density they are often outnumbered by other large predators such as spotted hyaena (*Crocuta crocuta*) and lion (*Panthera leo*) with low numbers of wild dog found even in such large reserves such as the Kruger National Park (Woodroffe and Sillero-Zubiri, 2012). The largest population of wild dog occurs in Botswana and Tanzania. Rabies has resulted in wild dog population declines in Kenya, South Africa and Tanzania in the 1990s. (Gascoyne et al., 1993; Hofmeyr et al., 2004; Woodroffe and Ginsberg, 1999).

2.6.3 History of rabies in wildlife in southern Africa

Rabies spread to Namibia in the 1940's from southern Angola and Zambia (Courtin et al., 2000). Black-backed jackal, dogs and bat-eared foxes are the most important carnivore hosts of rabies in Namibia. In the communal areas of northern Namibia the most important host is the dog, while in the central area where livestock ranching is most prevalent the black-backed jackal is the most important vector. Cases of rabies in jackal are seasonal with the highest during the dry season (July to September) when jackal travel over larger distances (Courtin et al., 2000). The incidence of rabies in wildlife peaks every 3 to 4 years (Courtin et al., 2000).The first case of rabies in kudu was described in 1977. The outbreaks of rabies in kudu follow the outbreaks in jackal but kudu also spread the virus horizontally to other kudus (Hübschle, 1988). Kudu account for the most reported rabies cases in Ethosha National Park in the north of Namibia (Laurenson et al., 1997a). The rabies virus found in kudu is closely linked to the canid biotype found both in jackal and dogs (Mansfield et al., 2006).

Wildlife rabies made up 4.5% of cases in Zambia between 1985 and 2004, with the side-striped jackal being the predominant wildlife species with 69% of all rabies cases in wildlife. It is responsible for rabies in the northern and central parts outside the national parks (Munang'andu et al., 2011).

Wildlife rabies in Botswana accounts for approximately 15% of cases, with higher numbers reported in the central and eastern parts of Botswana (Masupu et al., 1999). From 1980, rabies was also reported in kudu and other wildlife such as jackal (Masupu et al., 1999), while sporadic cases occur in yellow mongoose and small-spotted genet (*Genetta genetta*) (Swanepoel, 2004).

The yellow mongoose is a diurnal, burrow dwelling, social carnivore, which is widely distributed throughout southern Africa and usually found in the drier more open habitats. The highest population densities are found in central South Africa. They are predominantly insectivorous, preferring termites, beetles and locusts (Taylor, 1993). The yellow mongoose is responsible for up to 44% of wildlife rabies in South Africa (Nel et al., 2005). Other wildlife played a minor role in rabies in South Africa until it was noted in the black-back jackal population in the 1950's in northern South Africa (Mansvelt, 1956). It had spread in black-backed jackal from Zambia into South Africa and continued to spread south- and westwards (Brückner et al., 1978). From there it became established in the bat-eared fox populations of the western parts of South Africa (Thomson and Meredith, 1993).

There is greater variation in the *Lyssavirus* genotype 1 in southern Africa than in the rest of the world (Nel et al., 2005). Two biotypes are recognised in South Africa: the canid biotype, and the mongoose biotype previously known as the viverrid biotype (Nel et al., 2005). Phylogenetically the mongoose type is distinct from the canid biotype (Figure 2.4). There are five phylogroups of mongoose rabies identified in southern Africa: group 1 is found in slender mongoose in Zimbabwe, while groups 2 to 5 are found in South Africa in the yellow mongoose (Figure 2.5) (Nel et al., 2005). Mongoose rabies has been introduced to South Africa prior to the introduction of the canid rabies type by settlers and is well adapted to the mongooses (Van Zyl et al., 2010). The canid biotype is less infective to mongoose than the mongoose biotype with higher viral loads required to cause disease (Chaparro and Esterhuysen, 1993). The yellow mongoose and the slender mongoose (*Galerella sanguinea*) are capable of maintaining rabies virus infection.

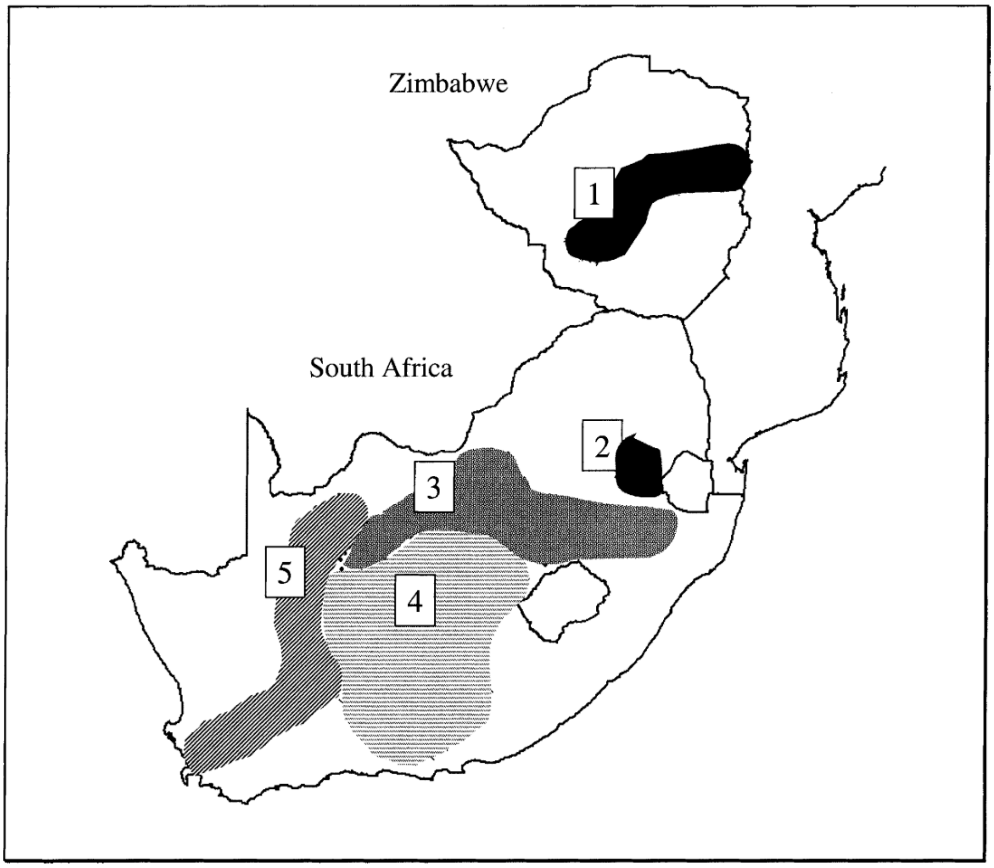

Figure 2.5: Geographical distribution of five mongoose rabies virus phylogroups identified in southern Africa (Nel et al., 2005).

Rabies usually results in fatal disease in wild carnivores, with the exception being in spotted hyaenas in the Serengeti ecosystem. In Tanzania 37% of spotted hyaena (*Crocuta crocuta*) showed evidence of exposure to rabies virus and only 13% of infected hyaena developed clinical rabies (East et al., 2001). Genotyping revealed a unique low-virulence strain in the hyaenas in that ecosystem (East et al., 2001). Spotted hyaena and wild dogs in Masai Mara (Kenya) had no antibody titres against rabies while 25% of wild dogs had rabies antibody titres in the Serengeti prior to vaccination campaigns (Gascoyne et al., 1993). Ethiopian wolves (*Canis simensis*) also had no rabies antibody titres prior to vaccination (Knobel et al., 2008). Antibody to rabies varied between jackal populations with 3-9% of jackal having positive titres in Namibia and Zimbabwe (Bellan et al., 2012; East et al., 2001; Foggin, 1988). The presence of antibody titres in wild carnivore populations seems to be linked to the sampling period, with antibody being more likely present during epidemics (Bingham and Foggin, 1993). No rabies antibody titres have to date been measured in free-ranging black-backed jackal in South Africa.

2.7 Jackal distribution and rabies epidemiology

Rabies in jackal is influenced by density of jackal, home range sizes and age structure of the population (Linhart et al., 1997). In Zimbabwe jackal densities of 1 to 1.4 jackal/km^2 are sufficient for the maintenance of rabies infection in the population without external introduction (Rhodes et al., 1998). The reported mean incubation period of RABV in jackals is 16 days (range: 10-31) and life expectancy of a rabid jackal is approximately 5 days (Bingham and Foggin, 1993). Jackals are moderately susceptible to rabies with 50 mouse intracerebral lethal dose ($MICLD_{50}$) resulting in disease (Bingham and Foggin, 1993). There are three jackal species of the genus *Canis* found in Africa: black-backed jackal, the side-striped jackal, the African golden wolf (in past classified as golden jackal) and one species in the Middle east and Europe the Eurasian golden jackal (Figure 2.6).

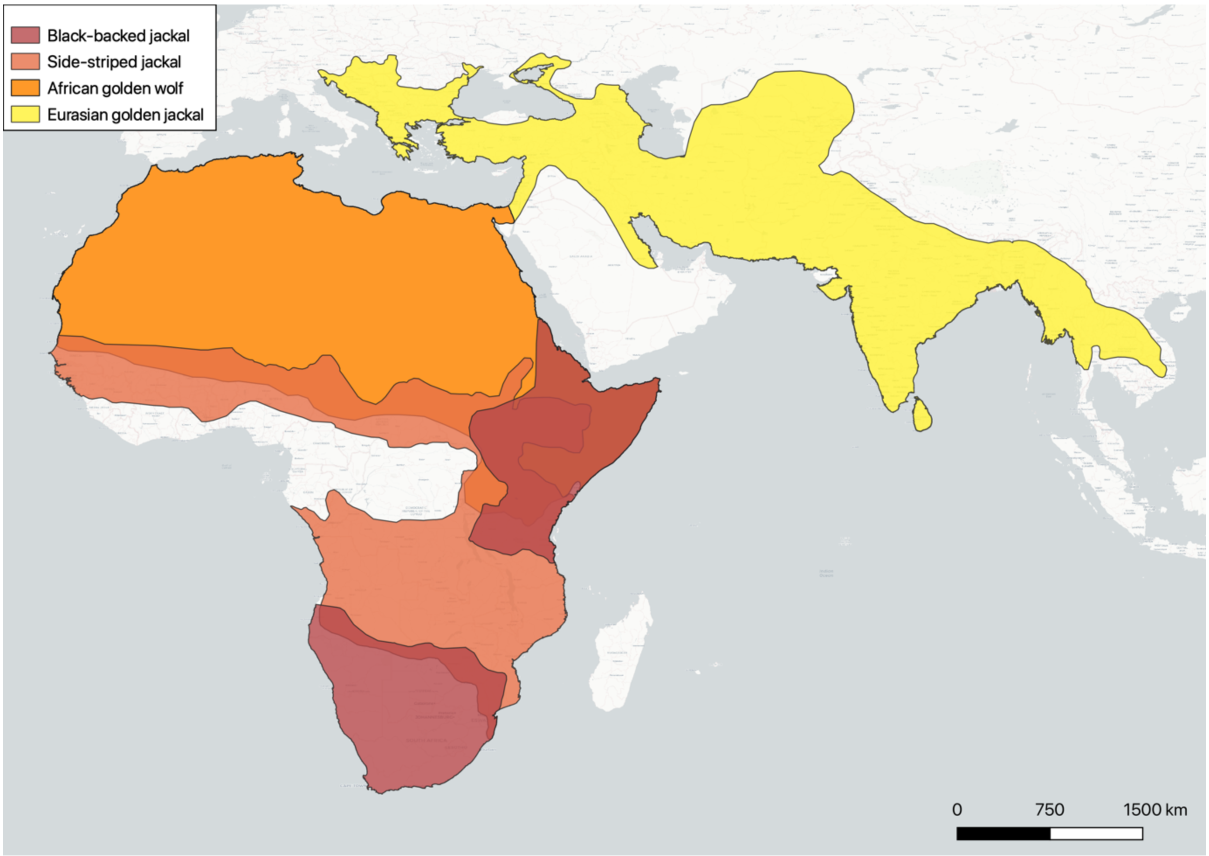

Figure 2.6: Distribution of Eurasian golden jackal (*Canis aureus*), side-striped jackal (*Canis adustus)*, black-backed jackal (*Canis mesomelas*) and African golden wolf (*Canis lupaster*) in Africa and Asia. Dark red areas show overlap in distribution between black-backed jackals and side-striped jackals and dark orange areas overlap between side-striped jackal and African golden wolf (Hoffman and Atickem, 2019; Hoffmann, 2014a, 2014b; Hoffmann et al., 2018).

2.7.1 Black-backed jackal

The black-backed jackal is smaller than the side-striped jackal, with the males weighing between 5 and 11.4 kg and females weighting between 5.9 and 10 kg (Skinner and Chimimba, 2005b). It has a characteristic black saddle that extends from the nape of the neck to the base of the tail.

Black-backed jackals are common in the drier areas and more open terrain. Territory size and home ranges vary greatly depending on abundance of food sources and land use. Home ranges between 1.5 km^2 to 50.1 km^2 have been reported in northern South Africa (Ferguson et al., 1983; Rowe-Rowe, 1982; Snyman, 2020). Home ranges of territorial pairs are usually less than for individuals (Ferguson et al., 1983). Smaller home ranges (mean 7.1 km^2) and greater overlap of territories are seen with greater food availability (Hiscocks and Perrin, 1988). Home ranges of 20 km^2 have been recorded in montane areas of Natal, where there is greater availability of small prey and lack of apex predators (Rowe-Rowe, 1982). Higher density of jackal are recorded in areas without apex predators (Yarnell et al., 2013). Jackal densities are highly variable between different habitats, and areas with the highest recorded density of 22 jackal/km^2 being in the Skeleton Coast in Namibia (Hiscocks and Perrin, 1988). The density of jackal in South Africa usually varies between 0.4/km^2 to 0.64/km^2 (Rowe-Rowe, 1982; Snyman, 2020).

Black-backed jackal are both diurnal and nocturnal. Their behaviour depends upon human disturbance, and in the more built-up areas jackal mainly move at night. In Botswana, jackal activity peaks between 17h00 and 22h00 and 05h00 and 08h00, with an increase in activity in the winter months (Kaunda, 2000). This is different to Namibia where most activity occurs between 09h00 to 18h00 with two distinct peaks in activity in morning and afternoon when it is cooler (Hiscocks and Perrin, 1988). Jackal are usually monogamous and pair bond for life. The offspring from the previous year often acts as helpers and this increases the survival of the latest litter (Moehlman, 1979). Reproduction is seasonal and in northern South Africa copulations usually take place during July to August. Gestation is 60 days and births are usually in August to October in South Africa and June to September in Botswana (Rowe-Rowe, 1975). Pups are weaned at 9 weeks of age and usually start leaving the den to forage with their parents at 14 weeks of age (Lombaard, 1971). Pup survival is usually low with only two pups per pair (mean litter size 5.4 pups per pair) surviving past 6 months (Rhodes et al., 1998). Jackals that do not manage to secure a territory do not usually survive past 3 years.

2.7.2 Side-striped jackal

Side-striped jackal are more nocturnal than black-backed jackal. Parturition is slightly later in the year than in black-backed jackal, from August to January (Skinner and Chimimba, 2005c). Side-striped jackal prefer open grasslands and their natural diet is made up mainly of small rodents and hares, with one third of their natural diet containing fruits (Atkinson et al., 2002). Home ranges decrease during whelping season and increase in the cold dry season, with the mean home ranges of side-striped jackal being larger then black-backed jackals in Zimbabwe (Loveridge and Macdonald, 2001). There are two peaks of rabies cases in Zimbabwe in side-striped jackals: one in the cool dry season (April-October) and the other in the hot and wet season (January-February) (Bingham et al., 1999). Side-striped jackal maintain rabies independently from dogs once rabies is introduced into the population in the north-eastern part of Zimbabwe (Bingham et al., 1999).

2.7.3 Eurasian golden jackal and African golden wolf

Recent literature places the African golden jackal into a different species the African golden wolf (*Canis anthus*) in contrast to the Eurasian golden jackal (*Canis aureus*) based on phytogenic analysis (Koepfli et al., 2015). Both species are phyleogenic very similar, therefore golden jackals reported in Serengeti are actually African golden wolves (Koepfli et al., 2015). Both *Canis anthus* and *Canis lupster* have been used by authors (Hoffman and Atickem, 2019) to describe the African golden wolf but Viranta et al. (2017) suggested that *Canis lupster* be used based on phylogenetic analysis. *Canis lupster* is used in this thesis to describe the African golden wolf.

The African golden wolf is larger than the side-striped jackal. It is widespread in North Africa and North-East Africa. It is phenotypically similar to the Eurasian golden jackal, which is found in central, eastern and southern Europe, the Middle East and parts of Asia (Arnold et al., 2012). In the African golden wolf parturition is recorded from December to March in the Serengeti. The breeding period varies throughout its distribution and is dependent on food availability (Negi, 2014). Rabies in jackals is increasing due to urbanisation of rural areas resulting in increased population density of jackals in some areas due to increased food availability (Yakobson et al., 2006). Rabies cases in Eurasian golden jackals occur in Israel and other parts of the Middle East (Linhart et al., 1997).

2.8 Rabies control measures

Effective rabies control programs require a “One Health” approach, with an integrated approach to rabies control in both dogs and wildlife (Be-Nazir et al., 2018). Surveillance of rabies cases is essential for an effective control of rabies (Townsend et al., 2013). Rabies in dogs can be controlled by mass parenteral vaccination campaigns dog population control and changes in legislation to enforce responsible pet ownership

(Conan et al., 2015; Velasco-Villa et al., 2017a). Culling strategies have been shown to be ineffective (Morters et al., 2013). For parenteral vaccination to be effective and to prevent the spread of rabies, it is generally accepted that 70% of the susceptible dog population needs to be vaccinated (Be-Nazir et al., 2018). This has been empirically established from observation of the relationship between rabies incidence and vaccination cover in dog populations worldwide (Coleman and Dye, 1996). Rabies associated death in humans can be significantly reduced by the provision of post-exposure prophylaxis (PEP). This, however, depends on the education of people to ensure that medical attention is sought soon after exposure. PEP is, however, only used in humans. The vaccination of dogs is generally more cost-effective than using PEP for humans as PEP are much more expensive in comparison to dog vaccinations (Brookes et al., 2018; Hampson et al., 2015).

Mass vaccination of dogs requires knowledge of RABV distribution within a country, knowledge of the dog population, access to dog populations and continuous funding to provide initial vaccination and to maintain the vaccination above the 70% requirement (Wallace et al., 2017). Several misconceptions reduce the effectiveness of rabies control in Africa, such as that rabies is only a problem in stray dogs and that it can only be controlled through culling or reduction in dog densities (Cleaveland et al., 2014). Wildlife vectors are, however, also significant in RABV transmission, especially in the southern African countries of Zimbabwe, Namibia, South Africa, Botswana and Zambia.

2.8.1 Population control in dogs

In dogs, culling strategies for population control are used, as well as non-lethal methods, such as population reduction through sterilisation, both chemically or surgically. The effectiveness of sterilisation techniques depends on population growth and influx of dogs from other areas. They have been effective in combination with vaccination campaigns in India and Africa by reducing population turn-over and reducing inefficient vaccinations (Fitzpatrick et al., 2016; Morters et al., 2014). Sterilisation campaign are often time consuming and expensive and have only limited impact on population size (LeRoux et al., 2018).

2.8.2 Population control in wildlife

Mass sterilisation techniques are not feasible for wildlife and culling strategies are usually employed to reduce animal numbers. Fox control measures by culling failed to control rabies in most of Europe (Wandeler, 1988). The number of foxes to be culled would need to be at least 70% of the population to bring it below the critical density of 0.2 foxes/km^2 in order to prevent the spread of rabies. Moreover, this is expensive and impractical to sustain for extended periods (Macdonald et al., 1981).

In the 1940's, control of rabies in North America in foxes, coyote and skunks was attempted via population reduction by trapping and poisoning (Rosatte et al., 2007). Strychnine and sodium monofluoroacetate were used for the poisoning of various wildlife species. The uptake of poison was variable within wildlife populations, averaging 30% with large variation between seasons (Linhart et al., 1993). Coyote getters (Human Coyote Getter, Inc., Pueblo, USA) were used in Zimbabwe and South Africa for the lethal destruction of jackal by firing a sodium cyanide bullet into the palate (Brand et al., 1995; Foggin, 1988). However, the effectiveness of coyote getters significantly decreased over time with jackal learning to avoid them (Brand et al., 1995). Large numbers of non-target species died due to exposure to poisoning. The destruction of carnivores leaves a vacuum, which results in an influx from the surrounding areas. The presence of natural barriers such as rivers and mountains will slow down influx of wildlife from surrounding areas (Rosatte et al., 1986), but the effects are only short term.

2.9 Vaccine development

The initial development of a rabies vaccine was done by Louis Pasteur in the early 1880s, via the passing of the virus from an infected dog into a rabbit and then injecting it back into dogs and observing if it was protective. Dogs vaccinated with the attenuated virus were then resistant to challenge with canid rabies virus (Dreesen, 2007). Pasteur found that serial intracerebral inoculation of monkeys with the canid rabies reduced the virulence of the virus further (Dreesen, 2007). The induction of virus neutralising antibodies are essential for the protection from rabies (Taylor et al., 1991). In 1927 the first rabies conference was held, and it was recommended that inactivated or attenuated viruses be used for dog vaccination (Dreesen, 2007).

Inactivated rabies virus vaccine requires a high virus load to be effective. The virus is either grown in a hamster kidney, suckling mouse brain or chicken embryo (CEO) (Dreesen, 2007). The virus is then killed either with UV light or beta propiolactone. Once inactivated, adjuvant such as aluminium hydroxide, aluminium phosphate or saponin (Yang et al., 2013) is added to improve the immune response to the vaccine (Dreesen, 2007).

Inactivated rabies vaccines can be divided into cell-culture vaccines and nerve tissue vaccines (Briggs et al., 2004). Attenuated nerve tissue origin vaccines from mice or lamb brain were used in Africa and America: however, this vaccine still resulted in death of vaccinated animals. The Flury low egg passage (x136) was then developed but the vaccine still resulted in rabies in young puppies, cats and cattle (Dreesen, 2007). Flury high egg passage (x205) was safer in cattle and puppies older than 3 months of age but still caused disease in cattle (Dreesen, 2007).

Three common tissue culture-derived rabies vaccines are Street Alabama Dufferin (SAD) which was adapted to hamster kidney cells, Flury and Kelv strain grown in CEO and Evelyn-Rokitnicki-Abelseth grown in porcine kidney cells (ERA) (Dreesen, 2007). Cell-culture vaccines are cheaper to produce and show fewer side-effects than nerve culture vaccines, and it was recommended by the WHO in 2018 to discontinue nerve culture vaccines (Be-Nazir et al., 2018).

Currently, inactivated, modified-live (MLV) and recombinant vaccines are available for domestic animals and wildlife. Modified-live strains have been adapted for oral vaccination, but since 2004 are no longer recommended for parenteral use as there is a risk of reversion to disease (Briggs et al., 2004). MLV vaccines are also more sensitive to temperature fluctuation, which can cause vaccine failure (Yang et al., 2013).

2.9.1 Parenteral vaccination

In the 1950s, parenteral vaccination resulted in control of rabies in the dog in Europe and Northern America. A variety of parenteral vaccines are currently available for use in domestic animals. There are no vaccines currently licenced for the use in wildlife except for ferrets. Vaccines availability depends on the licensing of the vaccine in the country, and the WHO provides guidelines for the production and safety of rabies vaccines (Office International Des Epizooties, 2008).

2.9.2 Oral bait vaccines

The first generation of oral rabies vaccine (ORV) used for fox control in Europe contained modified live virus. The rabies virus vaccines were made by passage of the virus through cells resulting in the SAD Bern, SAD B19, SAD P5/88, Vnukovo-32 or RV-97 vaccines (Müller et al., 2015). These vaccines had residual pathogenicity in rodents and carnivores, resulting in rabies in mice and a report of rabies in a cow in Romania, which was directly traced back to the SAD vaccine used in the area (Vuta et al., 2016). The SAD Berne vaccine also resulted in rabies in chacma baboons (*Papio ursinus*), making the vaccine unsuitable for the African continent (Bingham et al., 1992; Mähl et al., 2014). To improve safety, the second generation of vaccines were developed, including SAD VA1, SAG-1 and SAG-2, which had a deletion of certain pathogenic sites that improved safety in target species and non-target species. The SAG-2 (SAD Avirulent Gif) vaccine showed adequate protection in side-striped jackal, black-backed jackal and Ethiopian wolf (Sillero-Zubiri et al., 2016). Titres in side-striped jackal and black-backed jackal were adequate for 12 months post consumption (Bingham et al., 1999b).

The SAD strain has been used extensively in wildlife to control rabies outbreaks, deployed in blister packs stapled to chicken heads. The initial trial was conducted in a Swiss Alpine village where a 500-km^2 area was baited with between 12 to 20 baits per km^2 (Steck et al., 1982). This achieved a 60% coverage of rabies vaccinated foxes, measured through a tetracycline biomarker and resulting in a barrier against the spread of rabies at the time (Steck et al., 1982; Wandeler et al., 1988). Immunity levels of 50 to 60% in red foxes have been shown to be effective in preventing spread of rabies, compared to the 70% required in dogs (Cliquet et al., 2018; Rosatte et al., 2007).

The SAG-2 vaccine has been successfully used in a variety of wildlife species. Its safety was established in foxes and raccoons under controlled laboratory settings (Mähl et al., 2014). It has been used on a variety of carnivores to control rabies such as raccoon dogs, ferret badgers, side-striped jackal, black-backed jackal, golden jackal, Arctic fox, striped skunk, coyote and Ethiopian wolf (Bingham et al., 1999b; Cliquet and Aubert, 2004; Follmann et al., 2004; Hsu et al., 2017; Schumacher et al., 1993).

The third generation of ORV that were developed, were the recombinant virus vaccines with the rabies glycoprotein inserted into a viral genome (Rupprecht et al., 1993). The first one developed was the recombinant vaccinia rabies glycoprotein vaccine (VR-G®) (Müller et al., 2015). The vaccinia virus had been previously used to eradicate smallpox (Pastoret, 2002). The insertion of the rabies virus transmembrane glycoprotein into the vaccinia virus resulted in an adequate immune response in wildlife. VR-G® vaccine has been used in raccoons, foxes, coyotes and skunks in the USA (Maki et al., 2017). The VR-G® vaccine was, however, not effective in some wildlife species, such as the striped skunk, which lead to the development of a human adenovirus-rabies glycoprotein vaccine (ONRAB; Artemis Technologies, Guelph, Canada) (Slate et al., 2014). The ONRAB vaccine has been successfully used in raccoons and skunks in North America (Table 2.1). A recombinant canine adenovirus (serotype 2) rabies glycoprotein (CAV2-RG) vaccine has also been used for skunks and raccoons (Slate et al., 2011; Vos et al., 2017).

Table 2.1: Oral rabies vaccine used for various wildlife species from 1970 to 2018.

Vaccine name	Vaccine type	Bait matrix	Titre ($TCID_{50}$/dose)	Supplier	Target species
Fuchsoral	SAD B19	Fishmeal/coconut fat	10^8	Klocke Pharma Service, Weingartern, Germany (Linhart, 1993)	Red fox, raccoon dog
Altrofox 91	SAD P5/88	Meat/bone meal	10^7	Impfstoffwerk Dessau-Tournau GMBH (Linhart, 1993)	Red fox
Raboral V-RG®	recombinant vaccinia virus	Animal protein/fish oil	$>10^{6.5}$	Boehringer Ingelheim Animal Health, USA (Maki et al., 2017)	Raccoon, golden jackal, red foxes, raccoon dog
Rabifox oral	SAG1/SAG2	Fishmeal/beef tallow	10^8	Virbac, France (Linhart, 1993	Red fox
Lysvulven	SAD Berne	Fishmeal/beef tallow	10^7	Bioveta, Ivanovice, Czech Republik (Linhart, 1993)	Red fox
Kamark	Vnukovo-32-107	Fishmeal/beef tallow/dried milk	$>10^{5.6}$	Mevak, Nitra, Slovak Republik (Linhart, 1993)	Red fox Polar foxes
Ontario blister bait	ERA-BHK	Tallow/wax/chicken flavour	10^7	Langford, Guelph, Canada(Gilbert et al., 2018)	Striped skunk. raccoon, red fox
ONRAB Ultralite	recombinant human adenovirus	vanilla/wax/vegetable oil	$10^{9.6}$	Artemis Technologies, Guelph, Canada (Anonymous, 2009)	Striped skunk. raccoon, red fox

2.9.3 Factors affecting efficacy of oral bait rabies vaccines

The use of ORV in Europe has resulted in ten countries achieving national eradication of rabies in the country (Müller et al., 2015). ORV can be effective in the control of rabies in wildlife, but needs to be carefully selected for the species and habitat. The effectiveness of an oral bait vaccine depends on two factors: the uptake of the bait by the target species and the efficacy of the vaccine in the target species (Knobel et al., 2002). The most suitable vaccine type, bait type, time of distribution and interval of bait placement need to be investigated for each target species.

A variety of bait types have been tested in wildlife. The attractiveness of the bait depends on the preference of the target species, with omnivores such as raccoons responding to both cherry essence as well as fish based baits (Linhart et al., 2002). Gelatin and powered chicken egg are preferred by Indian mongoose (Berentsen et al., 2019). Carnivores, on the other hand often prefer local food items to novel ones. Ethiopian wolves preferred goat meat and grass rats (*Arvicanthis blicki*), their usual diet, over the commercial liver bait matrix (Sillero-Zubiri et al., 2016). Knobel et al. (2002) found chicken heads to be the preferred bait in African wild dog. Fishmeal polymer was accepted well by golden jackal and red foxes, with an uptake of 40-90% during the first night (Yakobson et al., 2006). Chicken heads have been effective baits in black-backed jackal in Zimbabwe with 70% taken within the first night (Linhart et al., 1993). Meat blocks and intestine have been used to deliver poison to black-backed jackals in the past (Linhart et al., 1993). According to peer reviewed literature bait preference has not been previously evaluated in black-backed jackals.

In foxes, a higher uptake of bait has been noted during autumn vaccination campaigns compared to spring, but season did not significantly affect uptake of bait in striped skunks (Linhart et al., 1993; Taylor et al., 2020). Bait uptake by non-target species, such as swine and deer, often increase during the winter months when less feed is available (Linhart et al., 1993).

Baiting density and distribution are greatly affected by bait uptake by target species. Increased baiting density has been shown to result in a higher proportion of sero-positive animals (Pedersen et al., 2019). Time of bait placement is also critical, with preferred foraging times varying between species. Raccoons often forage earlier in the evening compared to foxes (Steelman et al., 2000), so bait should be placed later in the evening if foxes are specifically targeted.

There is species variation in the response to oral rabies vaccine with striped skunks being very refractory to oral vaccination even with use of higher titres (Vos et al., 2017). Oral vaccine uptake is predominantly in the palatine tonsil and to a lesser extent in the oral mucosa after oral administration (Vos et al., 2017). The anatomical structure of the lymphoreticular system is similar in red foxes and skunks making it unlikely that absorption of vaccine from the oral cavity differs between those two species (Vos et al., 2017). The human adenovirus serotype 5 rabies glycoprotein vaccine (ONRAB) and the canine adenovirus serotype 2 glycoprotein vaccine (CAV2-RVG) have resulted in seroconversion of 88-100% of skunks with similar titres compared to V-RG® vaccine (Brown et al., 2014; Henderson et al., 2009). If recombinant vaccines are used, the pre-existing immunity to the vector vaccine is also important. Pre-existing immunity to canine adenovirus does interfere with vaccination success in dogs (Wright et al., 2013). To the author's knowledge, there are no antibodies to pox virus found in jackals.

The ORV needs to be chewed rather than swallowed to allow the virus to be in conduct with the palatine tonsil. Initially blister packs containing liquid vaccine, were stapled to chicken heads, which was later replaced by mixture of fish meal and fat that could be poured into a mould allowing easy distribution by aircraft (Bachmann et al., 1990). A variety of flavours and packaging has been tested for acceptance by various wildlife species (Maki et al., 2017; Rosatte and Lawson, 2001).

ORV has resulted in adequate antibody titres in both side-striped and black-backed jackals in a controlled environment using the SAG-2 vaccine (Bingham et al., 1999b). Israel used the Raboral VR-G® vaccine for the control of red foxes and golden jackals in the field. This resulted in a 71% reduction in rabies cases in vaccinated areas after a 5-year biannual baiting campaign (Yakobson et al., 2006). Field trials have not been conducted in black-backed jackals to date. The preferred bait type for uptake by black-backed jackals needs to be evaluated as well as the rabies antibody response to Raboral VR-G® vaccine and the development of antibodies in free-ranging jackals post vaccination in South Africa.

CHAPTER 3

Spatiotemporal patterns of rabies in South Africa: 1993-2019, with reference to the role of wildlife

Article in preparation

3.1. Summary

Rabies is a global viral zoonosis endemic to South Africa, resulting in fatal encephalitis in warm blooded animals, including humans. The loss of human lives and economic losses in rural areas through loss of livestock are substantial. A review was conducted of all confirmed rabies cases in animals in South Africa from 1993 to 2019, with a total of 11 701 cases identified to species level to assess the role that wildlife plays in the epidemiology of rabies. A spatiotemporal cluster analysis using a discrete Poisson space-time probability model, accounting for underlying estimated dog and livestock densities, identified 13 significant clusters ($p<0.05$). These included four long-term clusters lasting more than 8 years in duration and seven short term clusters lasting less than 2 years, with the remaining two clusters being of intermediate length. Outside of these endemic clusters, wildlife outbreaks in the remainder of South Africa were often less than one and a half years in duration most likely due to the rapid decline of wildlife vectors, especially jackals associated with rabies infection. Domestic dogs accounted for 59.8% of cases, with domestic cats (*Felis catus*; 3.2%), livestock (21.1%) and wildlife (15.8%) making up the remainder of the cases. Yellow mongoose (*Cynictis penicillata*) was the most frequently affected wildlife species, followed by bat-eared fox (*Otocyon megalotis*), black-backed jackal (*Canis mesomelas*), meerkat (*Suricata suricatta*) and aardwolf (*Proteles cristatus*). A shift was seen over time with a reduction in cases in yellow mongoose, and an increase in cases in black-backed jackal. Rabies in wildlife species followed different spatial distributions: black-backed jackal cases were more common in the north-western parts of South Africa, yellow mongoose cases more frequent in central South Africa, and bat-eared fox and aardwolf cases were more frequent in southern and western South Africa. Black-backed jackal, aardwolf and bat-eared fox cases were more common in the winter months (June to October). Clusters often spanned several provinces, showing the importance of coordinated rabies control campaigns across administrative boundaries, and high risk areas were highlighted for rabies in South Africa.

3.2 Introduction

Rabies is a viral disease caused by *Rabies lyssavirus* (RABV) belonging to the order Mononegavirales, family Rhabdoviridae, genus *Lyssavirus* (Amarasinghe et al., 2017). It is a negative stranded RNA virus resulting in fatal encephalitis in domestic wild animals and humans (Swanepoel et al., 1993). The number of human fatalities is thought to be underreported in developing countries, where rabies is often endemic, and is suggested to amount to economic losses equivalent to $4.7 billion worldwide (Hampson et al., 2015). In Africa, livestock losses are an important component of the cost of rabies (Knobel et al., 2005).

In South Africa, the control of rabies is difficult because of its endemic presence in domestic dogs in certain areas, as well as wildlife rabies in bat-eared fox (*Otocyon megalotis*), aardwolf (*Proteles cristatus*), yellow mongoose (*Cynictis penicillata*) and black-backed jackal (*Canis mesomelas*) (Swanepoel et al., 1993). Due to the variation in wildlife host ranges, rabies epidemiology often differs between the regions of South Africa. However, the domestic dog is still the most important species in the transmission of rabies, both in South Africa and the rest of the world (Morters et al., 2013; Sabeta et al., 2015). Dog rabies is endemic, especially in the densely populated rural areas of Limpopo (LP), Mpumalanga (MP) and KwaZulu-Natal (KZN) provinces of South Africa (Figure 3.1) (Zulu et al., 2009), and has persisted in KZN despite concerted efforts to control it by parenteral vaccination campaigns (Gummow et al., 2010). In 2011, 64% of dogs in KZN were reported to be vaccinated (Hergert et al., 2018), far higher than the 3.8% reported in 2004 (Gummow et al., 2010). The recommended minimum vaccination coverage is 70% for effective prevention of rabies outbreaks, but this was based on different environments and dog populations (Be-Nazir et al., 2018). Larger dog populations usually requiring higher vaccination coverage to prevent a major outbreak, although this could vary depending on the structure of the population (Coleman and Dye, 1996; Kaare et al., 2009). Failure of vaccination campaigns is thought to be due to the high density of dogs, especially in urban townships, and the high population turnover, with most dogs being younger than 3 years of age (Hergert et al., 2018).

Opinions differ on the role of the black-backed jackal in the maintenance of rabies infection, with several authors suggesting that it has no maintenance potential due to the high number of jackal required for the spread of rabies and low aggression in undisturbed jackal populations (Cleaveland and Dye, 1995; McKenzie, 1993). However, jackals are thought to be maintenance hosts for rabies under enabling environmental conditions such as high density and increased population turnover with larger distances travelled by jackals (Bingham, Foggin, Wandeler, & Hill, 1999; Foggin, 1985). In Zimbabwe, both the side-striped jackal (*Canis adustus*) and the black-backed jackal are considered to be important in rabies transmission, with more cases occurring in the side-striped jackal (Bingham, Foggin, Wandeler, & Hill, 1999). Density of jackal populations may thus be a key factor in the maintenance and spread of rabies. Zulu et al. (2009) found that in the northeast of South Africa RABV (the LP-1 phylogenetic cluster) was only found in jackals and persisted over a 5-year period, so black-backed jackals maintained rabies infection independently from dogs.

RABV and its molecular epidemiology have been extensively studied in South Africa (Coetzee and Nel, 2007; Ngoepe et al., 2009; Zulu et al., 2009). These studies provided insight into the spread of rabies within the dog population and also its spread into wildlife species such as the black-backed jackal. From 1980 to 2005, wildlife accounted for just 5% of rabies cases diagnosed at the Onderstepoort Veterinary Institute (OVI) (Knobel, Liebenberg, & Du Toit, 2003, Sabeta et al., 2007). The first reported case of rabies in black-backed jackal was recorded in 1947, when rabies also started to appear in the bat-eared fox population in South Africa (Thomson and Meredith, 1993). Between 1952 and 1990 there was a median of 6.5 reported rabies cases in jackal per year (IQR: 6-7.25 cases/year) (Bishop, 1988; Thomson and Meredith, 1993). There have been a few descriptions of the occurrence and distribution of rabies in wildlife but they have mainly focused on the molecular structure of the different rabies isolates (Sabeta, Mkhize and Ngoepe, 2011). Recently, the spill over of rabies from dogs to wildlife was investigated in the north eastern part of South Africa, the Lowveld region (Grover et al., 2018) but there has been no analysis of spatiotemporal trends of rabies in the rest of South Africa to date.

To achieve the goal of the World Organisation for Animal Health (OIE) and the Global Alliance for Rabies Control of reducing human deaths due to dog-associated rabies to zero by 2030. Thus it is essential to understand the spatial distribution and temporal patterns of rabies, as well as the hosts involved in the outbreaks in animals and the endemicity of rabies in any area (Rattanavipapong et al., 2019). In South Africa, rabies is a notifiable disease in humans and animals and surveillance is done at the national level by the National Institute for Communicable Disease (NICD) and the Department of Agriculture, Land Reform and Rural Development (DALRRD). All reports and samples from animals suspected of having contracted rabies are sent via the local State Veterinarian to the national rabies reference laboratory, where laboratory-confirmed cases are then recorded on the national database. The objective of this study was to provide a detailed description of the spatial distribution and temporal patterns of rabies from 1993 to 2019.

3.3 Materials and Methods

A rabies case was defined as a laboratory confirmed positive rabies result by direct fluorescent antibody test (Rupprecht et al., 2018). Data on animal rabies cases in South Africa from January 1993 to April 2019 were obtained from the national database maintained by DALRRD, and were aggregated by month and local municipality (DALRRD, 2019). Rabies cases were assigned to the centroid of the local municipality. In 1993, the nine current provinces and municipalities were established for South Africa. The nine provinces (first-level administrative division) of South Africa are divided into district municipalities (second-level) and local municipalities (third-level). The 2011 census demarcation of local municipalities was used for case aggregation throughout, as there were only minor changes in local municipality boundaries over the 27-year study period (Statistics South Africa, 2012). Centroids were obtained using the polygon centroid plugin tool through QGIS (Version 3.4.9; QGIS Development Team, 2018). Since there were such a low number of cases, where species were not specified they were excluded from the analysis (n=14, 0.001%). Microsoft Excel version 16.45 was used for data management and descriptive statistics.

Spatiotemporal cluster analysis was done with SaTScan Version 9.6 (Kulldorff 2009), using a discrete Poisson space-time probability model on monthly aggregated data and scanning for clusters with high rates within a circular window (Kulldorff et al., 2005; Kulldorff and Nagarwalla, 1995). Clusters with $p<0.05$ were considered significant. The maximum temporal window was set as 120 months (10 years) as epidemics in South Africa occur every 8 to 10 years (Bishop et al., 2010). The maximum spatial cluster size was set as 287 km (The radius at which Ripley's reduced second moment function K(r) is maximised and where the point pattern becomes independent (Dixon, 2014)). The window with the highest log-likelihood ratio (LLR) was defined as the most important cluster, and all significant ($P<0.05$) clusters were shown. The p-value of the LLR was estimated through 999 Monte Carlo simulations (Kulldorff, 2001).

For cluster analysis, species were aggregated into companion animals (dog and cat), livestock (equine, bovine, ovine, caprine or porcine) and wildlife (all wildlife species) and used as covariate in the cluster analysis. For companion animal (dog and cat) populations at risk, dog density was used and calculated using the 2011 human census data for South Africa (Statistics South Africa, 2012), at a ratio of 13 humans to 1 dog for rural and of 21:1 for urban areas (Akerele, 2014; Conan et al., 2017; Evans et al., 2019; Hergert et al., 2018; Knobel et al., 2005; Léchenne et al., 2016). Gridded livestock of the world (GLW3) was used to aggregate livestock numbers (cattle, sheep, goats and horses) per local municipality (Gilbert et al., 2010). Land cover suitability was used to adjust population density, with mine building, urban residential, informal settlement and commercial industrial areas categorized as unsuitable (Department of Enviromental Affairs, 2014). Population density per km^2 for dog and cattle are shown in Figure 3.1. Maps were generated using QGIS (Version 3.4.9). A relative risk map was created by calculating the risk for a particular municipality for rabies during the entire time period, comparing case numbers to the population size at risk. It was calculated as the observed divided by the expected within the cluster divided by the observed divided by the expected outside the cluster.

3.4 Results

There were a total of 11 732 laboratory confirmed animal rabies cases between January 1993 and April 2019 in South Africa, of which 11 701 cases were included in the SatScan analysis, since 31 records (0.3%) lacked location or species data (Table 3.1). The median annual number of confirmed animal rabies cases was 436 (range:264-673; IQR: 318-526). Dogs constituted the majority of cases (59.8%) and domestic cats were responsible for 3.2% of cases. In livestock, cattle were the most frequent with 15.8% of total cases, followed by goats (2.1%), sheep (1.3%) and equines (0.6%). Wildlife were responsible for 15.8% of all animal cases. Yellow mongoose was the most frequently affected with 35.5% of wildlife cases, followed by bat-eared fox (20.5%) and black-backed jackal (15.5%). Aardwolf represented 4.4% of wildlife cases and other mongoose species (7.4%) were sporadically reported (Table 3.1).

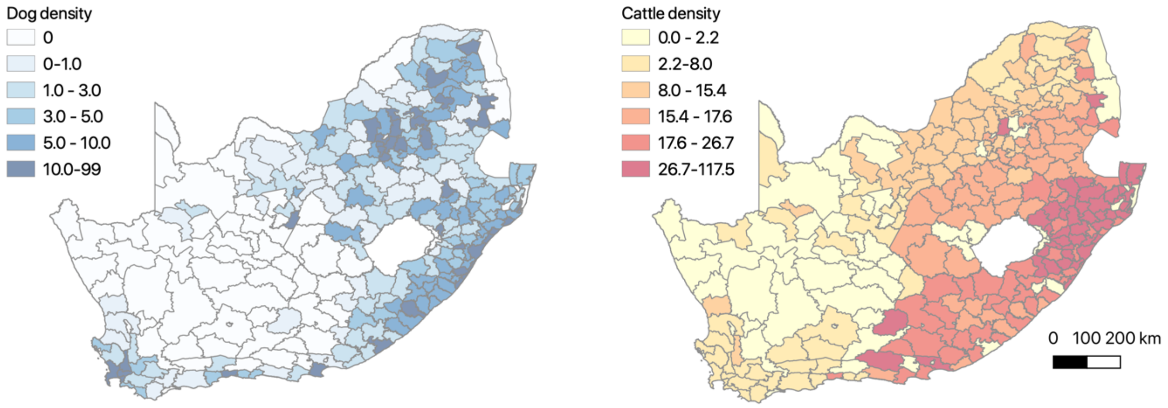

Figure 3.1: Estimated density of dogs and cattle per km^2 in South Africa based on the 2011 human population census and gridded livestock of the world 2010(Gilbert et al., 2010; Statistics South Africa, 2012). (The map was constructed using QGIS 3.4 using country and municipal boundaries)

The densely populated province of KZN (Figure 3.1) in the east of South Africa had the highest number of rabies cases by province, with 44% of the total cases from 1993 to 2019, followed by the Eastern Cape and MP. Wildlife cases were highest in the Free State (central South Africa), followed by the Northern Cape (Figure 3.2). Two cases have been found in avian species in this study (< 0.02 %).

The number of laboratory-confirmed cases of rabies in companion animals in South Africa between 1993 and 2019 peaked in 2007 with 499 cases reported (Figure 3.3). Companion animals showed a median of 283 cases per annum (IQR: 188-328), followed by 83 livestock cases per annum (IQR: 74-106) and 65 wildlife cases per annum (IQR: 39-93).

Table 3.1: Species associated with rabies in South Africa from 1993 to 2019, showing the percentage involvement of the species in overall rabies cases.

Species	**Scientific name**	**Cases**	**Percent**
Domestic		**9853**	**84.21**
domestic dog	*Canis lupus familiaris*	7001	59.83
cattle	*Bos taurus*	1925	16.45
domestic cat	*Felis catus*	379	3.24
goat	*Capra hircus*	261	2.23
sheep	*Ovis aries*	158	1.35
horse	*Equus ferus caballus*	72	0.62
farm animal	Species not identified	35	0.30
pig	*Sus scrofa domesticus*	20	0.17
avian	Species not identified	1	0.01
ostrich	*Struthio camelus*	1	0.01
Wildlife		**1848**	**15.79**
yellow mongoose	*Cynictis penicillata*	656	5.61
bat-eared fox	*Otocyon megalotis*	378	3.23
black-backed jackal	*Canis mesomelas*	287	2.45
meerkat	*Suricata suricatta*	114	0.97
aardwolf	*Proteles cristatus*	81	0.69
slender mongoose	*Galerella sanguinea*	67	0.57
marsh mongoose	*Atilax paludinosus*	31	0.26
small spotted genet	*Genetta genetta*	28	0.24
large grey mongoose	*Herpestes ichneumon*	24	0.21
Cape fox	*Vulpes chama*	21	0.18
Cape ground squirrel	*Xerus inauris*	16	0.14
African wildcat	*Felis lybica*	16	0.14
side-striped jackal	*Canis adustus*	14	0.12
honey badger	*Mellivora capensis*	13	0.11
striped polecat	*Ictonyx striatus*	13	0.11
common duiker	*Sylvicapra grimmia*	10	0.09
white-tailed mongoose	*Ichneumia albicauda*	9	0.08
caracal	*Caracal caracal*	8	0.07
African civet	*Civettictis civetta*	7	0.06
kudu	*Tragelaphus strepsiceros*	7	0.06
rock dassie	*Procavia capensis*	6	0.05
wild dog	*Lycaon pictus*	5	0.04
small-spotted cat	*Felis nigripes*	4	0.03
spotted hyaena	*Crocuta crocuta*	4	0.03
serval	*Leptailurus serval*	4	0.03
eland	*Taurotragus oryx*	3	0.03
Viverridae	Species not identified	3	0.03
banded mongoose	*Mungos mungo*	2	0.02
Burchell’s zebra	*Equus quagga burchellii*	2	0.02
lion	*Panthera leo*	2	0.02
Selous’ mongoose	*Paracynictis selous*	2	0.02
steenbok	*Raphicerus campestris*	2	0.02
Cape clawless otter	*Aonyx capensis*	2	0.02
large spotted genet	*Genetta tigrina*	1	0.01
Cape buffalo	*Syncerus caffer*	1	0.01
blue wildebeest	*Connochaetes taurinus*	1	0.01
brown hyaena	*Hyaena brunnea*	1	0.01
chacma baboon	*Papio ursinus*	1	0.01
dwarf mongoose	*Helogale parvula*	1	0.01
Rodentia	Species not identified	1	0.01

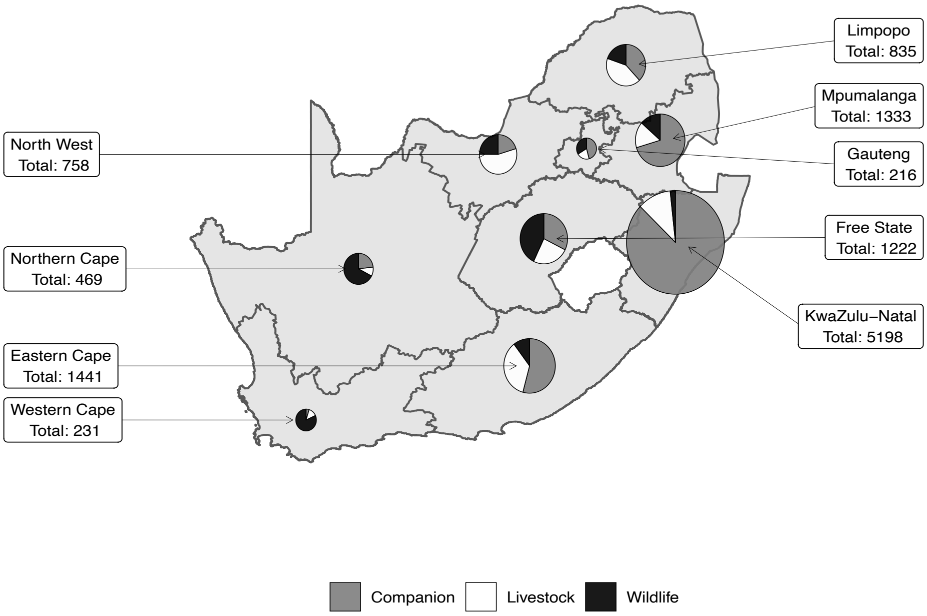

Figure 3.2: Map of rabies cases in South Africa 1993 to 2019 showing species categories involved by province. The diameter of the pie chart is proportional to the number of reported cases.

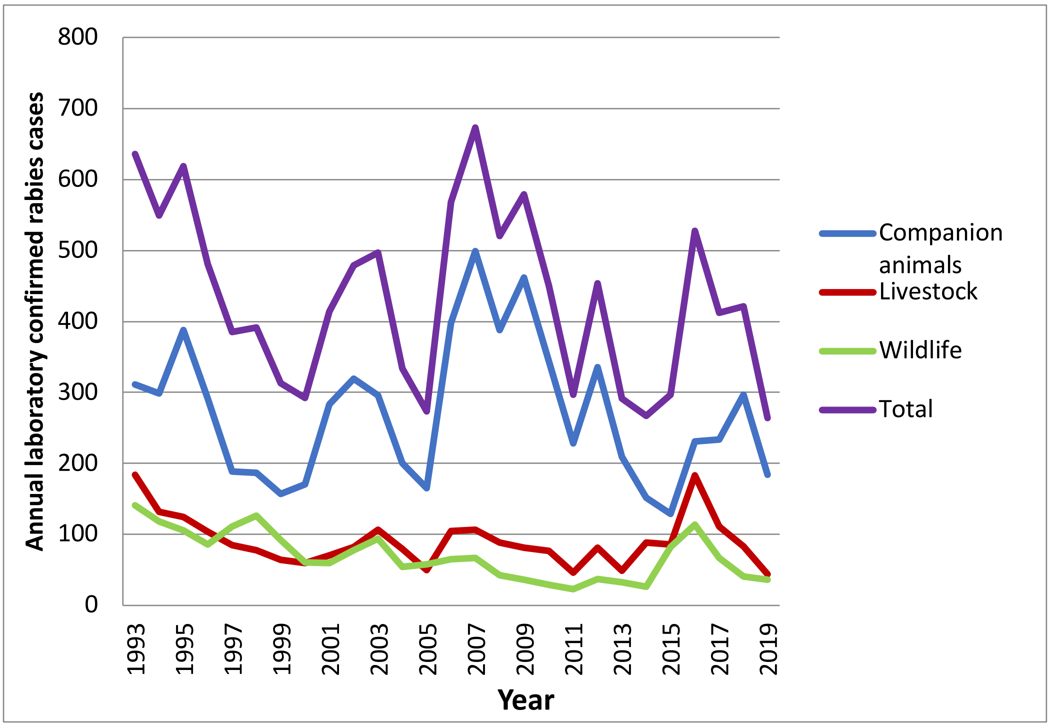

Figure 3.3: Annual confirmed rabies cases in companion animals, livestock and wildlife in South Africa from 1993-2019.

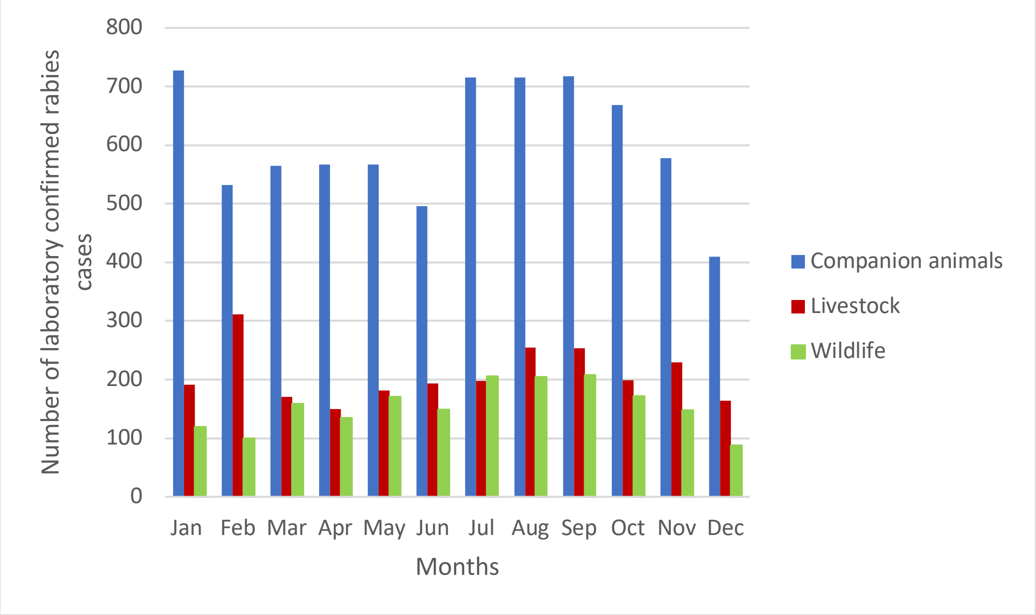

Figure 3.4: Monthly cumulative number of confirmed cases of rabies in companion animals, livestock and wildlife in South Africa from 1993-2019.

Monthly cumulative rabies cases for domestic animals over the entire study period showed no strong seasonal patterns (Figure 3.4), although canine cases increased in January and from July to October.

Spatiotemporal cluster analysis

Analysis for spatiotemporal clusters of rabies cases in South Africa from 1993 to 2019 revealed 13 significant clusters, varying considerably in duration from 1 month to 10 years (Table 3.2). There were four long term clusters (Clusters 1, 5, 9, 12) lasting more than 8 years, seven short term clusters lasting less than 1.5 years and two intermediate clusters (2, 8) lasting 4 and 8 years (Figure 3.5).

Table 3.2: Significant spatiotemporal clusters of rabies cases identified in South Africa, from 1993-2019.

Cluster	Start date	End date	Cluster duration (years)	No. of districts	Provinces	Log-likelihood ratio (p-value)	Observed cases	Expected cases
1	2003-02-01	2013-01-31	10	48	KZN	1522 (p <0.001)	2353	628.1
2	2008-07-01	2015-03-31	6.75	5	LP, MP	776.3 (p <0.001)	624	76.4
3	1996-01-01	1996-01-31	0.08	1	EC	312.2 (p <0.001)	65	0.2
4	2016-06-01	2016-11-30	0.5	8	NW, GP	198.8 (p <0.001))	114	7.9
5	1999-08-01	2008-05-31	8.83	1	LP	173.6 (p <0.001)	142	17.5
6	2016-01-01	2017-02-28	1.16	2	NW	158.3 (p <0.001)	66	2.3
7	2016-02-01	2016-02-29	0.08	1	FS	157.5(p <0.001)	35	0.1
8	1993-05-01	1998-03-31	4.91	2	LP	149.6 (p <0.001)	95	7.9
9	1993-07-01	2003-06-30	10	9	FS, NW	133.8 (p <0.001)	410	163.1
10	2006-02-01	2007-02-28	1.07	11	LP	131.9 (p <0.001)	145	25.75
11	2003-05-01	2004-09-30	1.42	1	MP	61.5 (p <0.001)	33	2.0
12	2010-04-01	2019-11-30	9.66	1	WC	57.1 (p <0.001)	65	12.0
13	2015-08-01	2015-08-31	0.08	1	NC	42.8 (p <0.001)	13	0.2

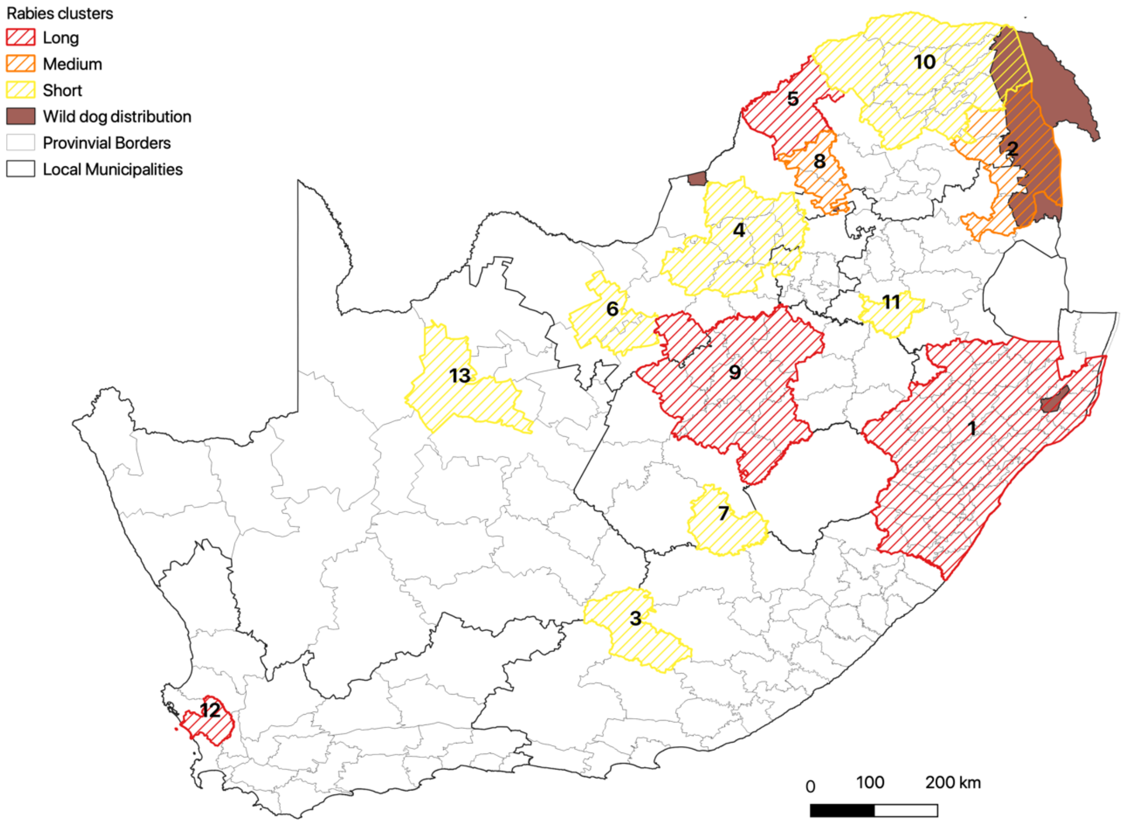

Figure 3.5: Spatiotemporal cluster analysis of rabies outbreaks in South Africa from 1993 to 2019 showing 13 significant clusters. The temporal length of the clusters is indicated by colour: yellow (short), orange (medium) and red (long). For details of each cluster refer to Table 3.2. (The map was constructed using QGIS 3.4 using country and municipal boundaries and wild dog

distribution with permission from IUCN red data list https://www.iucnredlist.org (Woodroffe and Sillero-Zubiri, 2012))

Clusters 1 and 2 were long and medium length clusters associated with dogs (Figure 3.5 and 3.6). Cluster 3 seemed to have started in domestic dogs and then became established in wildlife, marsh mongoose (*Atilax paludinosus*). There were dog and jackal cases prior to the start of cluster 4 but for the next 6 months, it mainly contained wildlife (black-backed jackal) and livestock cases. Cluster 5 seemed to display an endemic pattern associated with wildlife rabies - black-backed jackal and bat-eared foxes in particular - only occasionally involving domestic dogs. Cluster 6 was associated with cases mainly in black-backed jackal and livestock, with only a few domestic dog cases. Cluster 7 was a domestic dog cluster which spread to livestock. Cluster 8 was a medium length wildlife cluster associated with yellow mongoose and occasionally involving other wildlife species and domestic dogs. Clusters 9, 11 and 12 displayed a low grade, long term infection in wildlife, involving mainly yellow mongoose in clusters 9 and 11, and mainly bat-eared foxes in cluster 12. Cluster 10 was centred around domestic dogs but involved some black-backed jackals (n=9) and livestock. Cluster 13 was a short domestic dog outbreak of unknown origin as 3 months prior a common duiker was found positive but no other wild carnivores were involved.

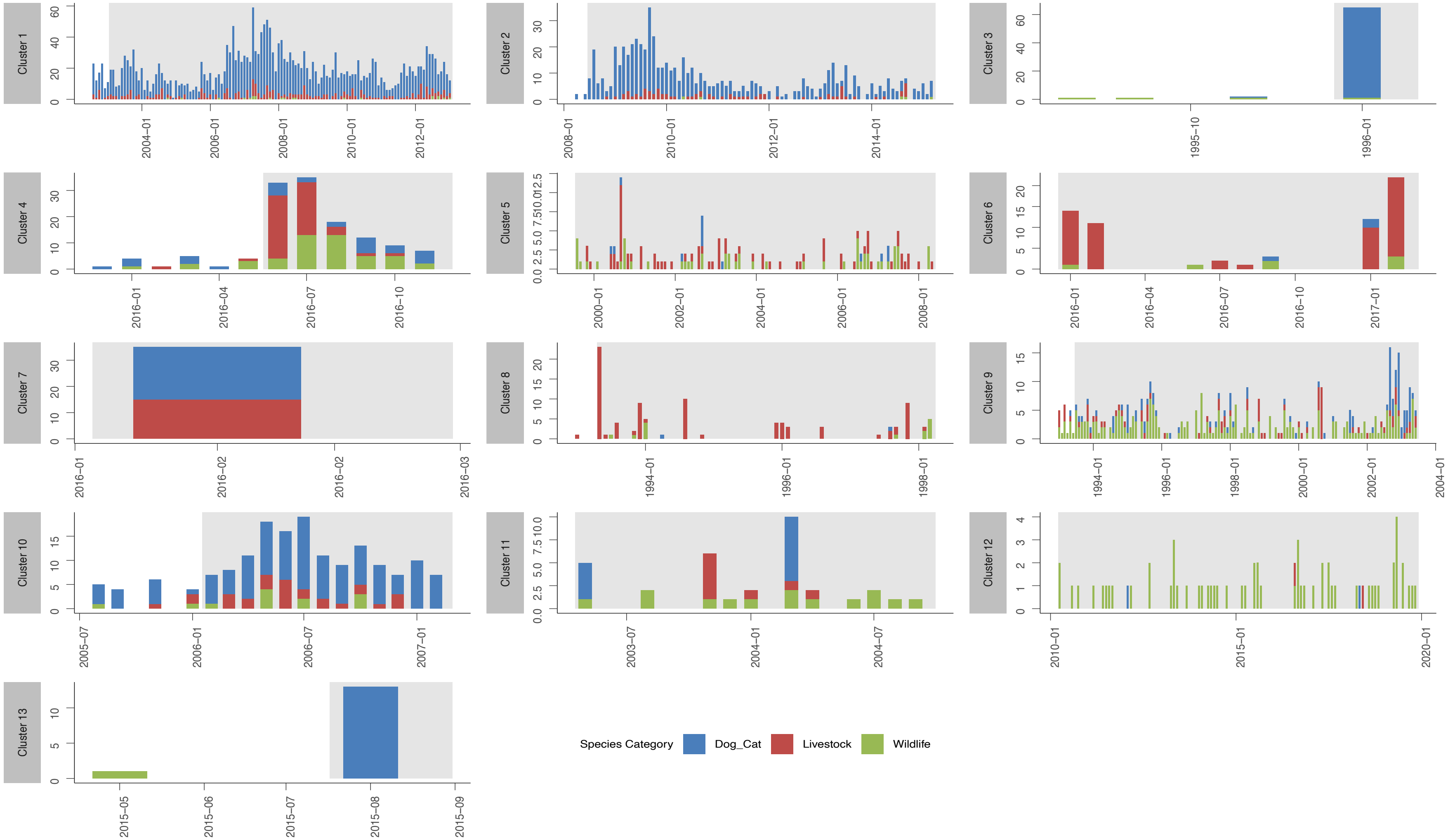

Figure 3.6: Analysis of rabies clusters in South Africa by species composition from 1993 to 2019. Clusters are shown in grey and any cases within six months prior to cluster in the same geographic area are also shown.

Wildlife rabies:

Wildlife species were reported in 9 out of 13 clusters. Yellow mongoose rabies case numbers have decreased since 1993 with the lowest reported annual incidence (n=3) in 2011 and 2014 and a slight increase in 2015 but staying with a median of 17 cases per annum (IQR: 7.5-38.5) over the entire period of analysis. Bat-eared fox case numbers were relatively stable with a median of 14 cases per annum (IQR: 10-17.5). Black-backed jackal had a median of 7 rabies cases per annum (IQR: 3.5-14.5) with the highest number (n=53) reported during the 2016 outbreak (cluster 4). Other wildlife species combined had a median of 20.5 cases per annum (IQR :13.5-25).

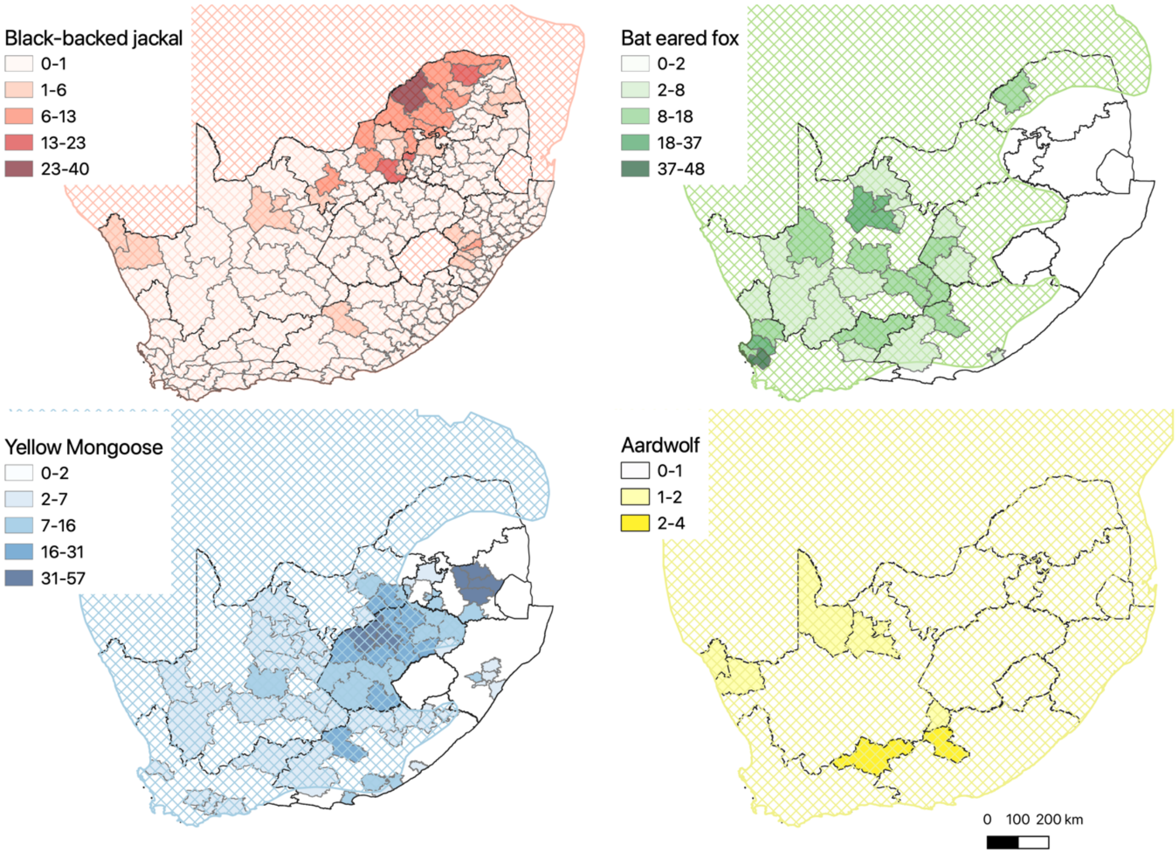

Figure 3.7: Distribution of number of rabies cases reported in wildlife in South Africa, 1993-2019, by local municipality showing the species distribution in chequered colour (Do Linh San et al., 2015; Green, 2015; Hoffmann, 2014c, 2014a). (The map was constructed using QGIS 3.4 using country and provincial boundaries and species distribution with permission from IUCN red data list https://www.iucnredlist.org.)

Black-backed jackal cases were seen in north-western region of South Africa (Figure 3.7). Bat-eared fox cases were important in the western half of South Africa. Yellow mongoose cases were common in the central part of South Africa. Some rabies cases in yellow mongoose were distributed outside the distribution for the species and might have been misidentified. Aardwolf rabies cases were seen in less populated areas of western South Africa (Figure 3.7).

Jackals were responsible for 15.5% of rabies cases in wildlife and constituted 2.6% of all rabies cases over the entire study period, varying between 0.2% and 10.0% per annum. The number of rabies cases in jackal appeared to show a cyclical pattern in South Africa, with an increase in reported cases approximately every 3 to 8 years (Figure 3.8).

In this study the black-backed jackal was responsible for 92% of reported rabies cases in jackals from 1993 to 2019. There were 274 reported rabies cases in black-backed jackal in South Africa from January 1993 to December 2019, and 13 cases in side-striped jackal: however some (n=6) of the latter occurred outside their distribution area, and were therefore likely misidentified black-backed jackals.

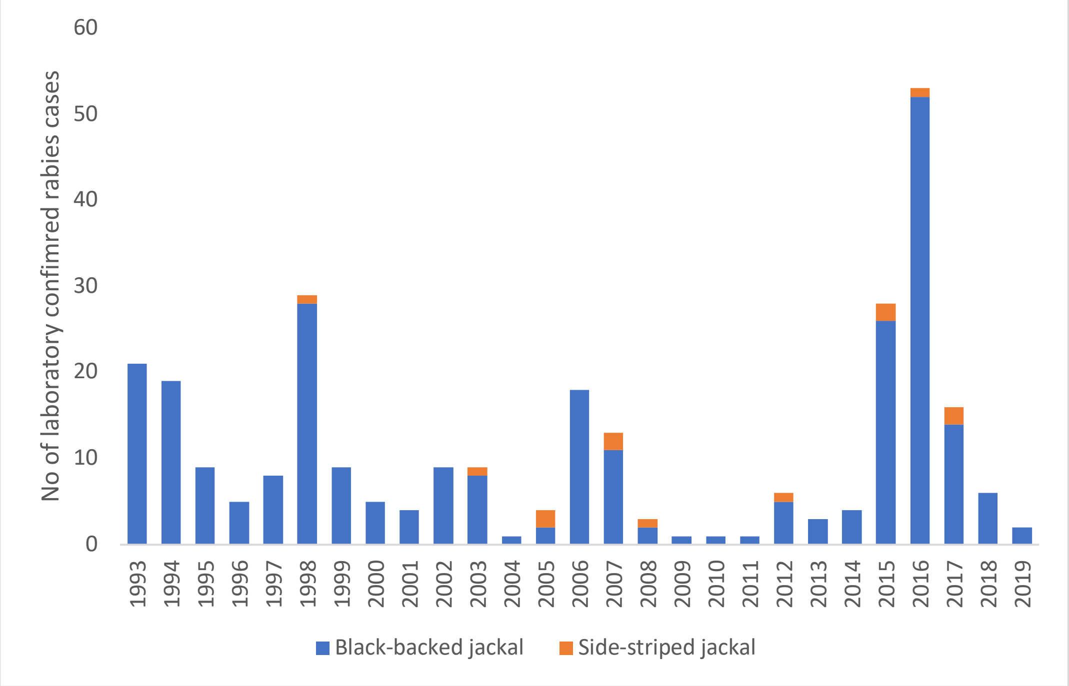

Figure 3.8: Number of rabies cases reported per year in jackals in South Africa from 1993-2019.

Monthly analysis of cumulative rabies cases showed two peaks in cases of rabies in black-backed jackals, with the main peak in July-August and a smaller peak in March. Aardwolf case numbers peaked in July. Bat-eared fox rabies cases showed the highest peak in July to October. Yellow mongoose case numbers were highest from May to November (Figure 3.9).

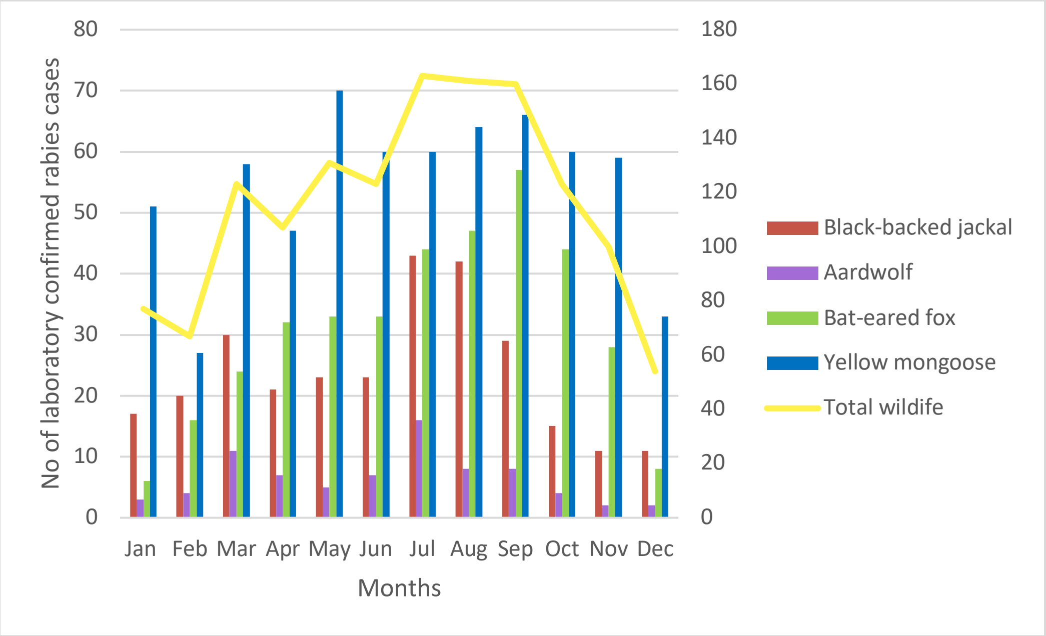

Figure 3.9: Number of confirmed cases of rabies in black-backed jackal, aardwolf, bat-eared fox and yellow mongoose in South Africa from 1993-2019, by month. Total wildlife case numbers are shown in right axis.

A relative risk map of rabies cases in South Africa showed that certain municipalities of False Bay, Uthungulu, uMashwathi, uMlalazi and Hibiscus Coast in KZN to have significantly higher risk of disease (Figure 3.10).

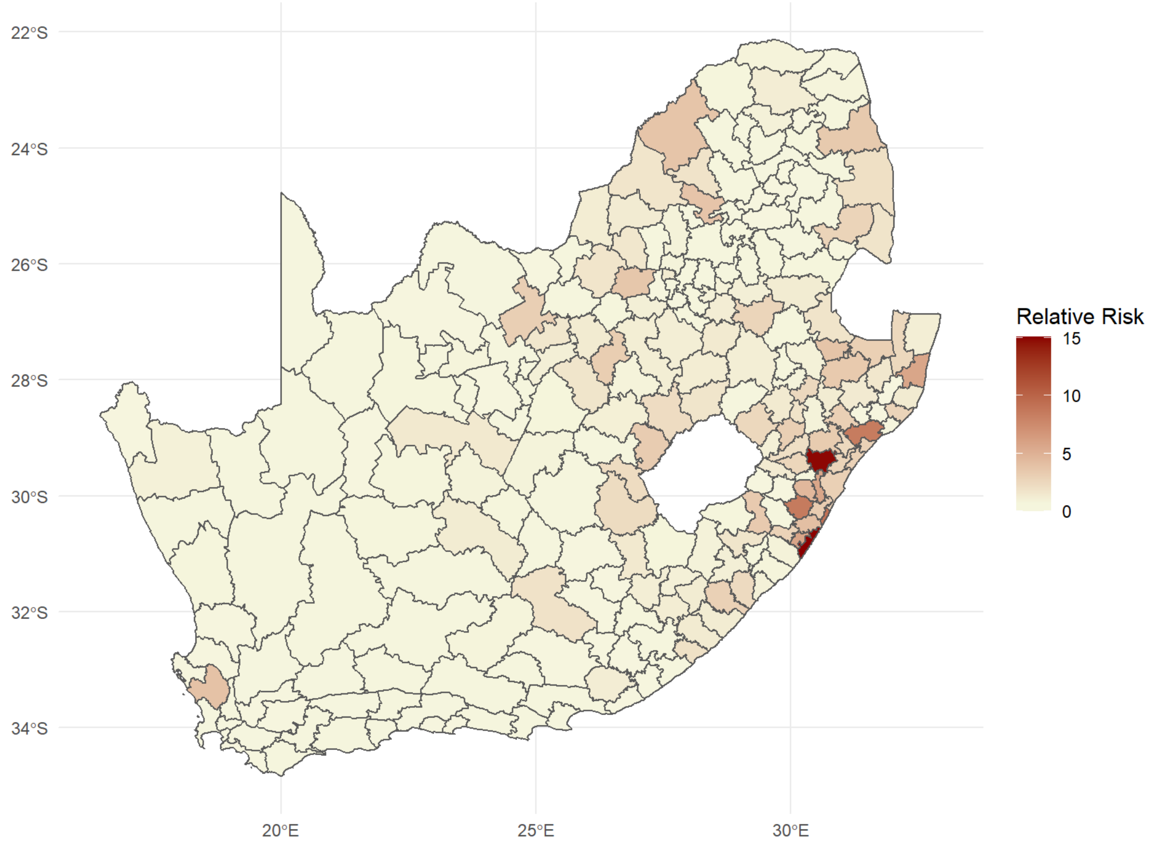

Figure 3.10: Relative risk map of rabies in domestic and wild animals for South Africa estimated from the 1993-2019 reported rabies cases.

3.5 Discussion

Rabies eradication remains a challenge in South Africa, due at least partly to its presence in both domestic dog and wildlife populations, and the spatiotemporal analysis shows that wildlife has been involved in the majority of observed clusters between 1993 and 2019. The failure of rabies control is thought to be more likely due to economic and logistical constraints (Perry, 1993). Mollentze et al. (2014) suggested that identification of core rabies clusters in endemic areas and targeted vaccination campaigns would allow control of rabies over larger areas. The spatiotemporal cluster analysis in this study highlights several areas where rabies is endemic across an extended area. Long term cycles in dogs are seen in the coastal region in the south eastern part (KZN) and in the eastern part of South Africa. These long-term clusters should be addressed with continued vaccination in the area, especially where there is a high canine density and turnover of dogs. Cluster 1, which was an endemic cluster in domestic dogs in KZN identified in this study since 2003, ended after 10 years due to massive dog vaccination campaign together with public education and awareness, showing the positive effects of a one-health approach on rabies cases. The campaign ended in 2014 and cases in KZN have been steadily rising although not yet established as a new cluster (LeRoux et al., 2018). The KZN province continues to have the highest number of rabies cases in South Africa, which poses a risk not only to the local human population but is also a threat to the rest of South Africa. The rabies outbreak in southern Johannesburg in 2011 was traced back to an infected dog from an endemically infected area in KZN (Sabeta et al., 2013).

The significance of wildlife in rabies transmission in South Africa is highlighted when analysing clusters of rabies cases takes into account population densities of domestic animals and livestock. Wildlife played an important role in the epidemiology of rabies in nine out of the 13 clusters identified either by being the likely index cases or maintaining the cluster. After yellow mongoose, black-backed jackal and bat-eared fox are the other two most important wildlife hosts in South Africa. Wildlife rabies cases occurred throughout South Africa, but wildlife cases depend on species distribution and density of wildlife in the area. In jackals, disturbance in the population, e.g. due to culling has been associated with an increased risk of rabies, while protected areas generally do not support rabies in jackal in South Africa (Bingham et al., 1999; McKenzie, 1993). Higher densities of black-backed jackal are found in the northern parts of South Africa in a variety of biomes but usually in the drier areas with rainfall below 1000 mm annually (Skinner and Chimimba, 2005b). Jackals accounted for 15.5% of wildlife rabies in this study, which is a large apparent increase from the 5% of wildlife cases reported between 1928 and 1992, despite wildlife cases overall having decreased from 41% of all between 1928 and 1992 (Swanepoel et al., 1993) to 15.8% between 1993 and 2019.

However, the 15.5% of rabies cases in jackals in this study is still below the 25% of confirmed cases that were attributed to side-striped and black-backed jackals in Zimbabwe (Bingham and Foggin, 1993). Difference between Zimbabwe and South Africa might be due to the fact that only black-backed jackals are involved in rabies epidemics in South Africa and cases in black-backed jackals are similar between the two countries in seasonal occurrence. Outbreaks of rabies in jackals in Zimbabwe have been linked to commercial farming practices in that country that favour rabies outbreaks, such as abundant food resources, high population turnover and lack of apex predators (Bingham, 2005; Swanepoel, 2004). Jackals are tolerated on commercial farms in Zimbabwe and there is suitable environment for breeding that allows jackal numbers to increase in those areas above the threshold of 1 jackal/km^2 that supports rabies outbreaks (Rhodes, Atkinson, Anderson, & Macdonald, 1998).

The 2016 rabies outbreak in the North West (NW) and Gauteng (GT) (cluster 4) was most likely initiated by dogs, indicated by cases prior to the start of the cluster, but once it was introduced into the susceptible jackal population it spread through the naïve population. The population of jackal in the area prior to the rabies outbreak was estimated at 0.64 jackal/km^2, but the outbreak resulted in a 80-93% reduction in a monitored jackal population in the north western parts of Gauteng (Snyman, 2020), similar to losses seen in other naïve African carnivore populations (Randall et al., 2006). Domestic dogs are often implicated in the introduction of rabies to wildlife (Hampson et al., 2007). The periodicity of rabies outbreaks in jackals in South Africa between 1993 and 2019 (every 3 to 8 years) is similar to those in Zimbabwe where they occur every 4 to 8 years (Bingham and Foggin, 1993). Outbreaks appear to have increased in frequency in South Africa, with outbreaks occurring 3 years after a previous one, an increase from the 5-year interval reported prior to 1993 (Brückner, 1993). This could reflect an increase in rate of contact between domestic dogs and jackals, particularly at the margins of rapidly expanding peri-urban areas. Particular attention should be paid to vaccination of dogs in such areas. In Zimbabwe up to half of rabies cases in dogs are associated with infection from jackals (Bingham et al., 1999a).

Rabies in wildlife seems to peak from May to November, the dry winter months, concomitant with a shortage of resources in most areas. Jackals and likely also other wild species, will increase their home ranges in response to reduced food availability (Ferguson, Nel, & Wet, 1983). In jackals specifically, rabies cases peaked in July and August, corresponding with the jackal's mating season, with a secondary peak in March associated with the dispersal of the young (Rowe-Rowe, 1975; Skinner and Chimimba, 2005b). The increased number of jackal and increased contact rates between individuals likely facilitate the spread of rabies. The same pattern is seen in aardwolf, with a peak in

July, corresponding to their short mating season in South Africa from the end of June to middle of July (Skinner and Chimimba, 2005d). Bat-eared foxes showed the highest peak in cases during July to October in this study. Bat-eared foxes forage in groups but groups split up prior to mating at the end of July and travel over greater areas, which can contribute to the spread of rabies (Nel, 1993). Food patches such as termites are shared between several family groups, which will further facilitate the spread of rabies (Nel, 1993). Rabies outbreaks in bat-eared foxes in the Serengeti were of short duration (Maas, 1993), which contrasts to this study where rabies persisted in the bat-eared fox population over several years as seen in clusters 5 and 12. Yellow mongoose cases peaked during the second half of the year, which may correspond to an increase in births and eviction of subadults from burrows due to food shortages (Taylor, 1993). Bovine cases peaked in February towards the end of summer (hot, wet season), which might be at least partly attributable to the increase in rabies in mongooses in January then spreading to livestock, such as was seen in clusters 8, 9 and 10. This was also seen in Namibia, where rabies cases are more frequent during the rainy season (January to June) in both wildlife and domestic animals (Hikufe et al., 2019).

Rabies virus transmission cycles are considered to be independent of each other in different wild canid and mongoose species (Sabeta et al., 2015), which is supported by the cluster analyses in this study, with only one of the clusters (Cluster 5) involving two wild canid species (black-backed jackal and bat-eared fox). The mongoose rabies virus biotype usually results in a dead end infection in other canids (Van Zyl, Markotter, & Nel, 2010).

Although this is a large data set over a long time period there might still be reporting bias, which potential could have influenced some of the findings. Rabies in wildlife is likely to be significantly under-reported since not all wild animals that die are evaluated for rabies, and during outbreaks not all cases of both domestic animals and wildlife are sent to a laboratory for diagnostic confirmation. The land use of an area might also have affected the reporting of rabies cases in wildlife with cases in communal farming areas potentially being underreported as there might be no transport available to the local state veterinary services (Hikufe et al., 2019). In Namibia the number of reports of rabies cases decreased both in domestic animals and wildlife the further they were from the state laboratory (Hikufe et al., 2019), while higher poverty in an area was directly related to reduced surveillance in Latin America (Arias-Orozco et al., 2018). So, there is a high likelihood that rural areas with high poverty did not report as accurately as more affluent areas. Public awareness in KZN has increased since 2014 due to targeted campaigns, which would have also improved reporting in the province. The combination of disease data with environmental surveillance can assist in guiding future interventions for South Africa making control strategies more effective.

3.6 Conclusion

Rabies control remains a challenge in South Africa due to the long-term persistence of endemically infected areas with high dog densities and low vaccination coverage, as well as the persistence of rabies in wildlife and the potential of wildlife species to perpetuate outbreaks. This is exacerbated by changes in land use and farming practices, as well as increases in human and dog populations. The location and duration of rabies clusters provides an indication of areas with high rabies occurrence and persistence in both dogs and wildlife. This could be used to identify and target areas of high risk with parenteral and/or oral vaccination of domestic animals and wildlife, as well as other risk mitigation measures.

CHAPTER 4

Oral bait preferences for rabies vaccination in free-ranging black-backed jackal (*Canis mesomelas*) and non-target species in a multi-site field study in a peri-urban area in South Africa

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4.1 Summary

Black-backed jackals (*Canis mesomelas*) are small meso-predators that occur in the wild and around cities and towns in southern Africa and have been associated with the spread of rabies in South Africa. Oral bait rabies vaccine has been used in Europe and the USA for the control of rabies in reservoir species. The effectiveness of an oral vaccination strategy depends not only on the efficacy of the vaccine but on the uptake of the bait in the target species. This study evaluated factors associated with the uptake of oral bait by free-ranging jackal and other wildlife species in a multi-site field study in Gauteng Province, South Africa. Three different baits were offered: commercial fishmeal polymer, pieces of red meat and chicken heads. Bait uptake was observed using camera traps and patterns of uptake assessed by multiple correspondence analysis and Cox proportional hazards models. In general, all the baits were well accepted with an uptake of 91%. Median consumption time of bait for jackal was 18 hours (IQR: 8-21 hours; range 7-66 hours) and for all other species it was 21 hours (IQR: 4-44, range 1-283). In species other than jackals there was a faster uptake in the winter months when less food was available, and the vegetation was sparse, whereas jackal showed no seasonal preference. Jackal consumed 20% of baits placed and took all three bait types but showed a clear preference for chicken heads if available (Hazard ratio (HR)=3.41; 95%CI: 1.16-9.99; p=0.025). Species other than carnivores preferred fishmeal polymer or red meat. Jackals showed no preference for time of day whereas herbivores and other species clearly preferred day; other carnivores preferred either day or night but not both, depending upon species. This study showed that chicken heads may be the preferred bait type for oral vaccination of black-backed jackal in this area, and that consideration should be given to placing bait during summer and at dusk, in order to minimize uptake by non-target species.

4.2 Introduction

Black-backed jackals (*Canis mesomelas*) are found throughout southern Africa in a variety of habitats, but are more often associated with open terrain (Skinner and Chimimba, 2005b). Due to their adaptability to different habitats and their exploitation of human introduced food sources, they are often found in close proximity to cities and towns (Ferguson et al., 1983; Kuhn, 2014). Immature jackals (<3 years of age) move over large areas; the average distance travelled varies depending upon the age of the jackal, with juveniles traveling further, up to 30 km having been recorded in one night (Ferguson et al., 1983). Once a territory is established mature animals tend to cover greater distances compared to immature animals but spend more time looping within their territory. The home range varies within different areas of South Africa with one individual having a home range of 244 km^2 over the winter months in the North West Province (Ferguson et al., 1983). The average home range in the North West was about 100 km^2; this is in contrast to Suikerbosrand Nature Reserve, south of Johannesburg, where 10 km^2 was the average territory size (Ferguson et al., 1983). The territory size is closely dependent on food availability and is usually occupied by a breeding pair, often with helpers from the previous litter. There is considerable overlap between territories in some areas (Ferguson et al., 1983; Hiscocks and Perrin, 1988).

Despite continued persecution, black-backed jackals continue to thrive in peri-urban environments around Johannesburg and Pretoria, the largest cities in Gauteng. The greater Johannesburg Metropolitan area has an estimated population of 10.5 million people and greater Pretoria 2.1 million people (United Nations, 2014). The Cradle of Humankind (a UNESCO world heritage site) lies approximately 50 km northwest of Johannesburg within the Magaliesberg Biosphere Reserve. The Cradle of Humankind consists of approximately 47 000 hectares (180 square miles) of privately-owned lands. The John Nash and Malapa Reserve is situated within the Cradle of Humankind (Figure 4.1).

There have been 3 outbreaks associated with jackal rabies in Limpopo in 1993/94, 1998 and 2006. In North West Province there have also been 3 outbreaks in 1997/98, 2003 and 2016/17 in jackal. There have been 2 major rabies outbreaks in Gauteng in the last

10 years. One outbreak was in dogs in the South of Johannesburg in the Soweto area in 2010/2011 and the other outbreak in 2016 centered around jackals in the Cradle of Humankind. Domestic dogs are responsible for most reported human rabies cases in KwaZulu-Natal Province, whereas in the rest of South Africa wild carnivores are the most important factors in the transmission of rabies (Gummow et al., 2010).Yellow mongoose (*Cynictis penicillata*), bat-eared foxes (*Otocyon megalotis*), aardwolf (*Proteles cristata*) and jackals are the most common wildlife reservoirs in South Africa, with black-backed jackal being the most important host in Limpopo and North West provinces (Gummow et al., 2010).

From January 2007 to October 2018, 127 cases of rabies were reported in Gauteng, of which 43 cases (34%) were in wildlife. The black-backed jackal was not a major component in these outbreaks until the most recent outbreak in 2016; in this outbreak, 48 cases were reported of which 28 (58%) were in jackal, 25 of which occurred in the Cradle of Humankind (P Geertsma, 2016, personal communication). The majority of cases were observed between June and August 2016, consistent with previous studies which reported a higher incidence of rabies in wildlife in the winter months (Gummow et al., 2010).

The relative importance of wildlife in rabies transmission has increased with the effective control of rabies in the dog population by massive parenteral vaccination campaigns. However, vaccination of domestic dogs on their own is ineffective in preventing rabies outbreaks if jackal together with other carnivores constitute a maintenance population for rabies independent of domestic dogs (Haydon et al., 2002). The 2016 Gauteng outbreak resulted in a total of 34 people being exposed to rabies from jackal, either directly or indirectly via infected livestock, and requiring post exposure prophylaxis (Directorate Animal Health, 2016). This highlights the importance of controlling rabies in the jackal population in and around Gauteng.

Diseases that persist in wildlife are often challenging to control. Controlling the disease is especially important if it poses a risk to public health (e.g. rabies) or livestock and wildlife conservation (e.g. tuberculosis) (Bhattacharya et al., 2011; Cross et al., 2007). Culling has been used to control wildlife diseases such as tuberculosis, although the relationship between disease and density of vectors is not linear (Woodroffe et al., 2008). However, due to the large geographic spread of rabies in meso-carnivores, culling strategies have only been effective in the short term (Cross et al., 2007) or not effective at all, as there is no simple relationship between rabies incidence and host population density (Morters et al., 2013).

Both parenteral and oral vaccination strategies have been used in the past as an alternative to culling strategies for control of infectious diseases in wildlife populations. Oral vaccination strategies allow large-scale distribution of vaccine to wildlife reservoir or hosts. Use of oral bait vaccine in the control of rabies has been highly effective in Europe, where the red fox *(Vulpes vulpes*) is the main wildlife reservoir, and has resulted in the eradication of rabies from some European countries (Müller et al., 2015). The red fox in Europe occupies a similar ecological niche to jackals in southern Africa (Rhodes et al., 1998).

The uptake of bait by non-target species is dependent on density of target and non-target species, habitat, food availability and type and presentation of the bait. Several oral bait vaccines have been used over time for the control of rabies in meso-carnivores such as domestic dogs, foxes, raccoon (*Procyon lotor*) and coyote (*Canis latrans*) (Cross et al., 2007; Steelman et al., 2000; World Health Organisation, 2007). All oral rabies vaccines used currently are either modified live or recombinant live vaccines. The World Health Organization (WHO) currently recommends two vaccines for oral use: Raboral VR-G® (Boehringer Ingelheim, previously Merial, USA) and SAG-2 (Virbac, Carros, France) (Office International Des Epizooties, 2008). Raboral V-RG® is a recombinant vaccine containing a vaccine vector with a rabies glycoprotein, is registered in the USA and Europe (Maki et al., 2017), and has been used in golden jackals (*Canis aureus*) in Israel. The SAG-2 vaccine is a modified live vaccine which has been genetically modified from the SAD vaccine (Virbac, Carros, France) by a change in two base pairs associated with virulence to make it safer. The SAD (Berne) strain of rabies vaccine has been used effectively to orally vaccinate both black-backed and side-striped (*Canis adustus*) jackals in captivity, producing adequate titres (>0.5 IU/ml) for up to 12 months. Oral rabies vaccines are routinely used in wildlife in North America and Europe but there has also been renewed interest in its use in dogs to improve overall cover in a population where parenteral vaccines have not been completely effective (Cliquet et al., 2018).

The effectiveness of an oral bait vaccine depends on two factors: the efficacy of the vaccine in the target species and the uptake of the bait by the target species (Knobel et al., 2002). When bait was presented in fishmeal polymer a 40-90% acceptance by red foxes and golden jackals was noted in the first night (Yakobson et al., 2006). Oral bait preference has been investigated in the Ethiopian wolf (*Canis simensis)* (Sillero-Zubiri et al., 2016); the wolves were offered a liver bait matrix (SAG-2Dog, Merial), goat meat, boiled goat intestine or grass rats (*Arvicanthis blicki*), their main prey, containing the rabies vaccine. There was a significant difference in uptake between the different baits, with the commercial bait not being taken and goat meat being the preferred bait (Sillero-Zubiri et al., 2016). Knobel et al. (2002) found chicken heads to be the preferred bait in African wild dog (*Lycaon pictus*). Fish meal polymer was well accepted by golden jackal in Israel with an acceptance rate of 100% (Linhart et al., 1997). Chicken heads have been effective baits in black-backed jackal in Zimbabwe with 70% taken within the first night (Linhart et al., 1993). Meat blocks and intestine have been used to deliver poison to black-backed jackals in the past (Linhart et al., 1993). However, to our knowledge, bait preference has not previously been evaluated in black-backed jackals.

The objectives of this study were to evaluate the preference of free-ranging black-backed jackals in the Cradle of Humankind, Gauteng, for chicken heads and meat compared to the commercial fishmeal polymer bait, to assess the relative uptake of the different bait formulations by non-target species, and to identify factors associated with bait uptake in the various species.

4.3 Materials and Methods

Ethical approval:

This study was approved by the Animal Ethics Committee of the University of Pretoria (V127-16).

Study Area:

The John Nash and Malapa Reserve is situated in the center of the Cradle of Humankind UNESCO world heritage site and contains 76.3 km^2 of protected area within the Magaliesberg Biosphere Reserve in Gauteng Province, South Africa (Figure 4.1). The topography is hilly, with open, rocky grassland and scattered trees and bushes, and thickly vegetated gullies and ravines. Rainfall occurs in summer, mainly between October and March, whereas winters are cool and dry. The reserves have been under study via camera traps continuously since 2009 (Kuhn, 2014), which showed that the reserves are home to numerous black-backed jackals.

Study design:

This placebo trial was conducted at 11 camera locations on the John Nash and Malapa Reserve from November 2016 to August 2017. Lynx Optics Ranger Trail Cameras (Lynx Optics, Bromhof, South Africa) were used to monitor the locations (Figure 4.2). Each camera location was baited with a fishmeal polymer and another randomly selected bait type, either a piece of red meat (beef or horse) or a chicken head, to compare preference. Cameras were placed at each location and were checked weekly during the trial periods. Each camera was triggered with an infra-red motion sensor: 3 pictures were taken in quick succession with a 5 second delay before the camera would take the next potential three-picture burst.

Baits were placed in front of the camera traps at least 60 cm apart from each other, in clear view of the camera trap, and no further than 5 m from the camera trap to ensure visibility during nighttime. All baits were placed around midday. Each camera location was baited with either meat or chicken on alternative weeks and the other bait was always fishmeal polymer. The two baits were distributed at each camera location. Baits were not placed if one of the cameras was malfunctioning at the time of placement and could not be repaired or replaced at the time. Each camera location was baited 1 day per week over 5 weeks during the summer months (November to January) and 1 day per week for 6 weeks during the winter months (June to August) (Figure4.4). Due to the inaccessible terrain in many part of the study area, camera locations could not be evenly distributed, but were selected based on proximity to roads and known jackal occurrence at the camera location (Kuhn, 2014). The camera locations were checked every 7 days and the presence or absence of bait was recorded; if bait was still in place it was left in situ, otherwise a new bait of random type was placed. Location, season, date, time period until the bait was taken, time of day, species that consumed the bait and kind of bait taken were recorded based on the data captured by the camera trap and analyzed.

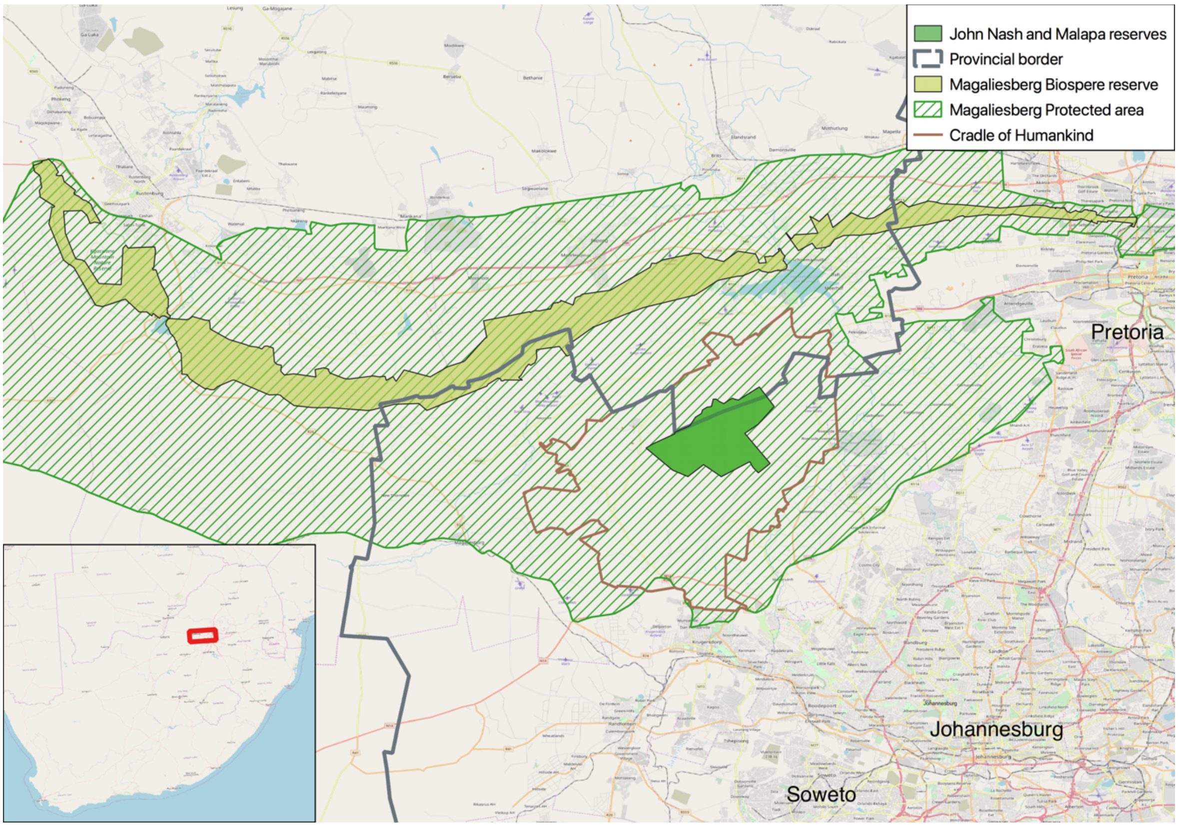

Figure 4.1: Map of the Cradle of Humankind showing the Magaliesberg Protected Environment, Cradle of Humankind and John Nash and Malapa Reserve, Gauteng Province, South Africa. (The map was constructed using QGIS 3.4 using country boundaries and protected area boundaries downloaded and used with permission from protectedplanet.net.)

Bait description:

The placebo was a plastic sachet containing 2 ml sterile water, measuring approximately 0.5 cm x 2 cm x 6 cm and was presented either in the original fishmeal polymer, or in a piece of meat, or in a chicken head and neck. The fishmeal polymer measured approximately 3.3 x 3.3 x 2.1 cm and weighed 23 g. For the meat bait, the plastic sachet was placed in a piece of red meat (horse or beef) approximately 10 x 5 x 5 cm in size, which was closed with absorbable suture material to ensure the sachet did not fall out. For the chicken heads, obtained from Barlet Poultry, Muldersdrift, a sachet was inserted into the mouth of the chicken and pushed into the neck area to ensure that it did not dislodge easily (Figure 4.3). Selection of bait types was based on reports that fish meal polymer has been well accepted in golden jackal and chicken heads were well accepted in black-backed jackal in Zimbabwe (Bingham et al., 1995; Linhart et al., 1997). Intestines were not used as bait due to the poor acceptance of it by jackal at vulture restaurants close to the study area (unpublished data).

Figure 4.2: Camera trap locations in John Nash and Malapa Reserves in the Cradle of Humankind, Gauteng Province, South Africa. (The map was constructed using QGIS 3.4 using country boundaries and protected area boundaries downloaded and used with permission from protectedplanet.net)

Statistical analysis:

Species was classified as jackal, other carnivores, herbivores and others; season was either summer (November to January) or winter (June to August); and bait type was fishmeal, chicken head or meat. Time of day was classified as day, night or twilight and calculated on the sunset and sunrise in Gauteng during the study period. In winter twilight hours are shorter (total 2 h 50 min) compared to summer (total 4 h 50 min). Categorical variables were cross-tabulated, and proportions were compared using Fishers' exact test. Median time to bait uptake was compared between species and bait types using the Mann-Whitney *U* test. Using all observations for which the species taking the bait could be identified, multiple correspondence analysis was used to identify patterns amongst variables (bait type, species, time to taken, season and location) and scatterplots of the normalized coordinates of variable categories in two dimensions were produced; for this, time until bait was taken was categorized into quartiles (<5 hours, 5-20 hours, >20-40 hours and >40 hours). Kaplan-Meier survival curves were generated for each species category, including a combined non-jackal species category, to show their relative preference for the different baits, indicated by their rate of bait uptake. Cox proportional hazards regression models were used for each species category to estimate the associations of bait type, season and camera location with time to bait uptake, while controlling for confounding amongst the three variables. Stata 14 (StataCorp, College Station, TX, U.S.A.) was used for statistical analysis; significance was assessed at p<0.05.

Figure 4.3: Types of baits offered to jackal and evidence of consumption. A: Fishmeal polymer; B: red meat; C: chicken head and neck and D: punctured sachet confirming uptake

4.4 Results

A total of 195 baits were placed, of which 186 were monitored via camera traps; nine recordings were lost when cameras were destroyed during a veldt fire. Overall, there were 102 fish polymer, 32 chicken heads and 52 meat pieces placed. All camera locations were baited with each bait type. 100 baits were placed over 5 weeks in the summer months and 86 baits over 6 weeks in the winter months. The overall uptake of bait was 87% (170/195) if the baits destroyed in fire are included. 25% of baits were taken within 4 h of bait placement, 50% were taken within 21 h and 75% were taken within 44 h. Of the 186 placements it was possible to identify the species eating the bait in 105 cases (55%) (Table 4.1).

Table 4.1: Bait consumption and median time to consumption by different species in a multi-site field study in South Africa

Species	**Scientific name**	**No of baits taken**	**%**	**Median time to consumption (h)**	**IQR***
Herbivore					
Eland	*Taurotragus oryx*	2	2	9	9 -9
Greater kudu	*Tragelaphus strepsiceros*	4	4	8.5	4 - 55
Nyala	*Tragelaphus angasii*	1	1	6	6 -6
Blue wildebeest	*Connochaetes taurinus*	3	3	48	29-52
Carnivore					
Black-backed Jackal	*Canis mesomelas*	21	20	18	8 - 21
Caracal	*Caracal caracal*	1	1	16	16 - 16
Honey badger	*Mellivora capensis*	1	1	17	17 - 17
Brown hyaena	*Parahyaena brunnea*	7	7	32	8-129
Serval	*Leptailurus serval*	4	4	21.5	2 - 41
Slender mongoose	*Galerella sanguinea*	4	4	13	4 - 21
Yellow mongoose	*Cynictis penicillata*	2	2	1	1 - 1
Other					
Warthog	*Phacochoerus africanus*	27	25	22	4-30
Chacma baboon	*Papio ursinus*	13	12	48	5 - 98
Vervet monkey	*Chlorocebus pygerythrus*	1	1	1	1 - 1
Dung beetle	Scarabaeidae	2	2	38	18-58
Pied crow	*Corvus albus*	12	11	2.5	1 - 5
Total		105	100	21	4-44

* Interquartile range

Figure 4.4: John Nash and Malapa reserve during summer (left) and winter (right) months

Warthogs, jackals and primates were responsible for the most bait consumption (Table 4.1). Jackal consumed 21 (20%) of the total baits identified, with other carnivores eating 19 (18%) and the remainder being taken by herbivores, primates and other species. The uptake was fastest when taken by pied crows, which would consume bait within an hour of placement. The median time to consumption for jackal was 18h, not significantly different from the median for other carnivores (16h) ($p=0.577$). Of the 21 baits consumed by jackal (Table 4.2), chicken heads (38%) and fish polymer (38%) were taken equally despite three times as many fish polymers being placed, and meat was consumed less often (24%). Chicken heads were taken only by scavengers and carnivores, including jackals, with the exception of one taken by a warthog.

Table 4.2: Consumption of three bait types by black-backed jackal and other species categories in in a multi-site field study in South Africa

Species	Number (%) of baits consumed			
	Fish polymer	Chicken head	Meat	Total
Jackal	8 (38%)	8 (38%)	5 (24%)	21
Other carnivore	13 (68%)	2 (11%)	4 (21%)	19
Herbivore	6 (60%)	0 (0%)	4 (40%)	10
Other	27 (49%)	7 (13%)	21 (38%)	55
Total ID	54 (51%)	17 (16%)	34 (32%)	105

Figure 4.5: Camera trap showing the fire that destroyed several cameras at John Nash and Malapa Perserve, South Africa.

The multiple correspondence analysis (Figure 4.6) showed jackals grouping with night and twilight in the first dimension (x-axis) and with chicken heads in the second dimension (y-axis). Jackals were most commonly found at the plains site (PL), the camera location in the center of the combined reserves (Figure 4.2), while herbivores were common at various camera locations, particularly RD. For season, winter tended to group with shorter uptake times and summer with longer uptake times.

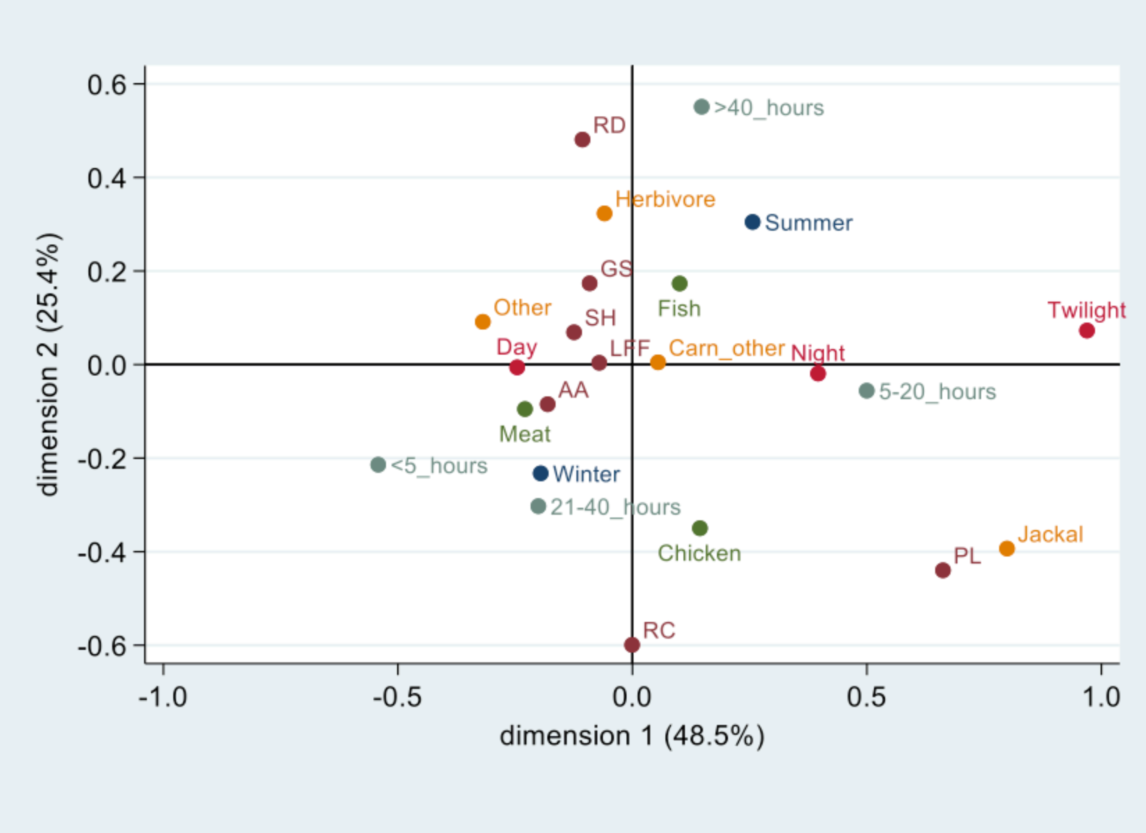

Figure 4.6: Multiple correspondence analysis of factors affecting bait uptake by black-backed jackal and other species categories in a multi-site field study in South Africa

When comparing survival times, jackals showed a clear preference for chicken heads (Figure 4.7) whereas other species showed no clear preferences (Figure 4.8), except that herbivores did not consume chicken heads (Figure 4.8B). The Cox proportional hazards models (Table 4.3) showed that jackals clearly consumed chicken heads more readily than fishmeal (Hazard ratio (HR)=3.41; p=0.025), although in comparison to meat the difference was not significant (HR=2.3; 95%CI: 0.7-7.6; p=0.174). Season was not associated with consumption of bait by jackal (p=0.574), but other species showed increased rate of consumption of bait in winter (Table 4.3).

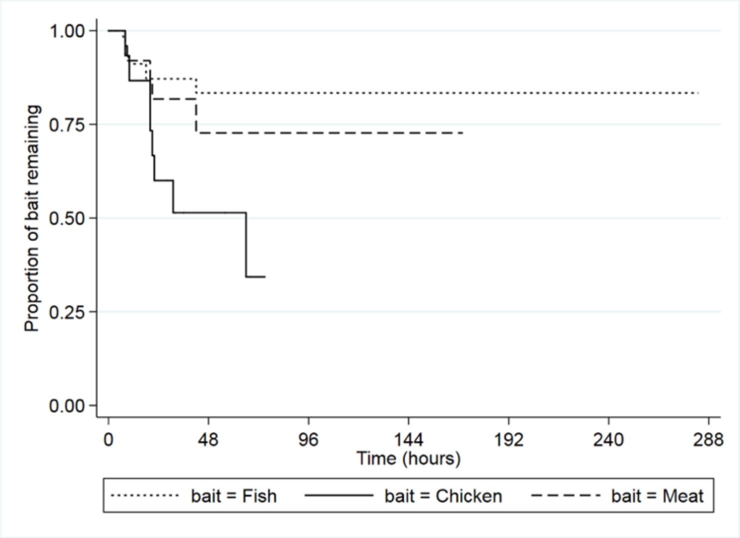

Figure 4.7: Kaplan-Meier survival curves showing time until different bait types were taken by black-backed jackal in a multi-site field study in South Africa

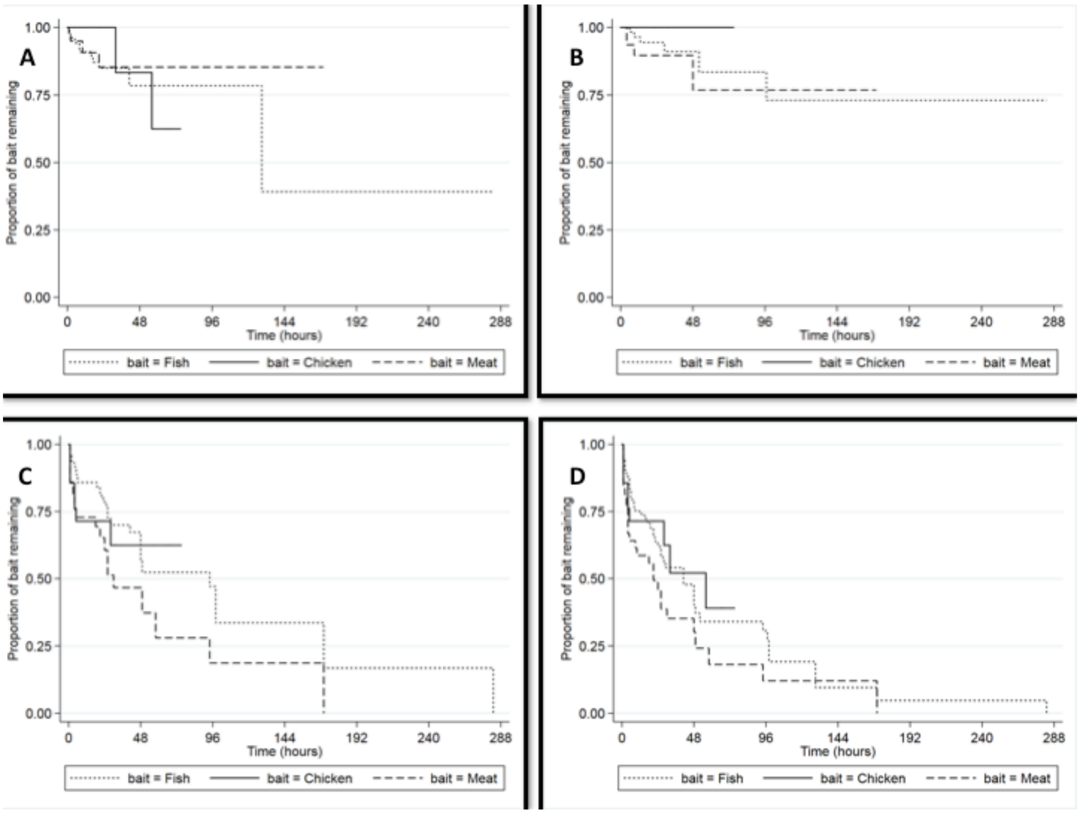

Figure 4.8: Kaplan-Meier survival curves showing time until different bait types were taken by species other than jackal in a multi-site field study in South Africa.

A: Other carnivores; B: Herbivores; C: Other species; D: All species other than jackal.

Table 4.3: Cox proportional hazards models of associations of time to bait being taken with bait type, season and camera trap location, for black-backed jackals and other species categories in a multi-site field study in South Africa

Species category	Variable and level	Hazard ratio	95% CI (HR)	p-value
Jackal	Bait			
	Fishmeal	1*	–	–
	Chicken head	3.41	1.16, 9.99	0.025
	Meat	1.48	0.48, 4.59	0.495
	Season (Winter vs. summer)	0.75	0.27, 2.06	0.574
	Camera trap location (7 sites)	–	–	0.003
Other carnivore	Bait			
	Fishmeal	1*	–	–
	Chicken head	0.62	0.13, 2.90	0.544
	Meat	0.97	0.30, 3.16	0.956
	Season (Winter vs. summer)	5.74	1.86, 17.7	0.002
	Camera trap location (7 sites)	–	–	0.939
Herbivore	Bait			
	Fishmeal	1*	–	–
	Chicken head	0	–	–
	Meat	1.8	0.45, 7.59	0.39
	Season (Winter vs. summer)	11.19	2.05, 60.91	0.005
	Camera trap location (7 sites)	–	–	0.017
Other species	Bait			
	Fishmeal	1		
	Chicken head	1.02		
	Meat	2.1	0.43, 2.46	0.956
	Season (Winter vs. summer)	2.87	1.58, 5.22	0.001
	Camera trap location (7 sites)	_	_	0.098
All species other than jackal	Bait			
	Fishmeal	1		
	Chicken head	0.70		
	Meat	1.76	0.33, 1.49	0.357
	Season (Winter vs. summer)	3.77	2.31, 6.17	0.000
	Camera trap location (7 sites)			0.047

4.3 *Discussion and Conclusion*

To our knowledge this is the first published study of oral bait uptake and preference in black-backed jackals, an important vector of rabies in southern Africa. Placing monitored bait stations scattered throughout the habitat of the target species is expected to provide a good indication of bait uptake by that species. In this study the baits were well accepted overall, with 20% being taken by jackals, 18% by other carnivores and 62% by other species. Several factors associated with bait uptake were identified which may help in designing baiting strategies to target jackals for oral rabies vaccination while reducing uptake by non-target species. In a high number of cases the species taking the bait could not be identified due to camera failure, or movement of camera by baboons, veldt fire and storms. This negatively affected the power of the study by reducing the effective sample size; however, it should not have introduced bias, with the possible exception that consumption by baboons may have been slightly underestimated if they moved the camera prior to taking the bait.

The problem of non-target species consuming bait was investigated when widespread use of oral bait was initiated in Europe for the control of rabies in red foxes (Brochier et al., 1989). Bait uptake by non-target species is usually associated with carnivores and scavengers (Knobel et al., 2002). In trials in the USA no evidence of uptake of fishmeal polymer by white-tailed deer was observed (Hanlon et al., 1989), which is in contrast to the present study in which 10% of baits were taken by herbivores. The higher uptake by herbivores in this study may be due to the John Nash and Malapa Reserve being managed and fenced game reserve. Whereas jackal, small carnivores and warthog will commonly pass under fences, ungulates do not, therefore maintaining a stable high herbivore density, and potentially leading to food scarcity during the dry winter season. This is supported by the fact that rate of bait uptake by herbivores was strongly associated with season, occurring almost exclusively during winter. Warthog were the largest consumer of bait; this might be related to their abundance, with an estimated 300 warthog in the reserve. Warthogs are a common species in large parts of South Africa, even outside protected areas, and therefore our results may also be applicable outside protected areas.

Jackals showed a clear preference for chicken heads, indicated by their faster rate of uptake of chicken heads compared to fishmeal polymer and meat, although the latter was not significant, likely due to inadequate sample size. This is consistent with the findings of Knobel et al. (2002), where chicken heads were the preferred bait in African wild dog compared to fish or liver flavoured wax blocks or chicken mince. Linhart et al. (1993) reported an uptake of up to 70% of chicken heads by jackal during the first night of placement although no comparison with fishmeal polymer was made in this study. Chicken heads may therefore be better for targeting jackals as there is also less loss of bait to non-target species; this is supported by our finding that both red meat and fishmeal polymer were far more likely to be taken by competitor species than were chicken heads. However, chicken heads are more labour intensive as they need to be prepared individually and freshly while the fish polymer is more hygienic and easier to handle and store.

Design of an oral rabies vaccination strategy should consider the period of vaccine viability in relation to the expected rate of bait uptake by the target species. In this study the uptake of the bait was generally good with most bait being consumed within 40 hours of placement. The SAG-2 and V-RG® vaccines have been reported viable at 37°C for up to 42 hours and 168 hours respectively (Maki et al., 2017; Massen et al., 1996). The stability of the vaccine to direct sunlight has also to be considered in South Africa, as the SAG-2 matrix has been shown to melt in direct sunlight, in contrast with the V-RG® vaccine (Yakobson et al., 2006). However, the vaccine itself should not be exposed to direct sunlight when it is put into baits such as chicken heads or meat.

Timing of vaccine placement can assist with greater uptake of vaccine by target species. It has been suggested that bait uptake will be increased in winter months when less food is available to carnivores (Maki et al., 2017). Loveridge and MacDonald (2001) suggested vaccination of jackals before the mating season (April to June) and before the pups become independent (October to January). Maternally derived antibodies in domestic dogs will protect pups up to 42 days of age, but pups are susceptible to rabies after that time period, unless protection is boosted via vaccination (Chappuis, 1998). In the Cradle of Humankind jackal pups are usually born around mid-September to the beginning of October (Snyman, personal communication) which may make August and September appear to be suitable months for vaccine distribution, as pups are not independent, and food is still not as abundant, and bait therefore more readily consumed. However, our study indicates that uptake by non-target species is increased in winter months whereas uptake of bait by jackal is not significantly affected by season, suggesting that bait placement during summer months may be more efficient for targeting jackal. There is a risk of pups not being exposed to vaccine as the vaccine strain is usually destroyed by gastric acid in regurgitated food provided by their parents (Papatheodorou et al., 2018). The pups need to be old enough to consume bait themselves (Bruyère et al., 2000). This again suggests that vaccination during summer, once pups become independent, may be more effective.

In Europe baiting twice annually is advocated but research has shown that as long as one individual eats one bait and seroconverts, and 50 to 70% of target population is immunized, it will prevent a rabies outbreak (Rosatte et al., 2007). Ideally vaccines should be distributed initially twice yearly to ensure a maximum protection of target species (Maki et al., 2017) but obviously this is more labour-intensive and costly than once annually. When considering the cost of a vaccine campaign versus its effectiveness, our study would suggest that, in our study area, a single annual vaccination may be most effective if given in late December/January. Clearly, further research, including studies of duration of vaccine-induced immunity in jackals, is needed to determine the optimum timing and frequency of vaccination.

In this study only three bait types were compared, whereas there may be other bait types that might be more attractive to jackal. In Africa there will always be numerous carnivorous and omnivorous competitor species, making it unlikely that a bait type will be found that is attractive only to jackals. Nevertheless, more research may be needed into bait development and placement strategies to minimize uptake by non-target species. However, it is worth noting that all mammals, including most competitor species in this study, are susceptible to rabies and may also benefit from oral rabies vaccine.

Another limitation of this study is that, for logistical purposes, all baits were placed at around the same time of day, at midday; therefore, the effect of bait placement at different times of day could not be assessed. To our knowledge this has also not been investigated in previous studies; although a loss of 10% of bait to beetles and millipedes has been noted if bait was not consumed by jackal during first night of placement (Linhart et al., 1993). The effect of time of day on bait consumption by non-target species will vary between regions and seasons depending on the particular non-target species present in the area. In this study there were clear differences between species in the time of day when the baits were consumed. Numerically the biggest competitors for bait uptake were warthogs, baboons and crows, all of which invariably consumed the baits during the day, whereas jackals showed little preference. Therefore, this study suggests that placing bait at dusk or at night may largely eliminate these species as competitors and more effectively target jackals. This would still leave the other carnivores as competitors; however, they occurred in lower numbers, and in addition most of them may also be involved as potential rabies vectors and could therefore also be considered target species in a rabies vaccination campaign.

Another limitation of this study, leading to loss of statistical power, was that a substantial amount of data was lost, firstly due to loss of cameras because of fires (Figure 4.5) and flooding, and secondly, in many instances the species consuming the bait could not be identified. The latter could have either been due to camera failure, loss of image due to movement of cameras by baboons, or consumption of bait by small animals, such as rodents, that did not trigger the camera.

In conclusion oral rabies bait is suitable for targeting black-backed jackal in South Africa. Placement of bait should depend on area and non-target species present in the area. Ideally bait placement should be done bi-annually and January/February and July/August might be the best time periods to achieve highest exposure to target species. Bait should be placed in late afternoon early evening to eliminate competition with non-target species such as warthogs and antelope.

Antibody response to Raboral VR-G® rabies vaccine in captive and free-ranging black-backed jackals (*Canis mesomelas*)

Article in preparation

5.1 Summary

Rabies is a zoonotic disease that remains endemic in large parts of southern Africa due to its persistence in wildlife and domestic dog vectors. The black-backed jackal (*Canis mesomelas*) is primarily responsible for rabies outbreaks in northern South Africa. Two trials were used to test antibody response of the oral rabies vaccine Raboral V-RG® in black-backed jackal in captive and free-ranging situations, respectively. In captive jackals 10/12 (83%; 95%CI: 52-98%) seroconverted after single oral bait vaccination. Captive jackals (n=9) had adequate antibody titres (>0.5 IU/ml) at 4 weeks (median: 2.05 IU/ml; IQR: 0.6-5.7) and 12 weeks (n=10; median: 3.5 IU/ml; IQR: 1.5-8.3) and maintained antibody titres for up to 48 weeks (n=3; median: 3.4 IU/ml; IQR: 2-6.3) above the recommended 0.5 IU/ml. Four sites were baited with Raboral V-RG® vaccine for free-ranging, wild jackal, using fishmeal polymer and chicken heads. Three of the sites were in the northern part of South Africa (John Nash and Malapa Reserve, Cradle Nature Reserve and Lion and Safari Park) and one on the central plateau (Golden Gate National Park). Baiting was done during the winter months (July to September). In northern South Africa baits were distributed by hand or from vehicle at average baiting density of 3.6 baits/km^2 compared to 0.12 baits/km^2 in central South Africa. This resulted in 4/11 (33%; 95%Cl:11-69) of jackals trapped between 3-12 months after baiting having antibody titres compared to 5/7 (71% ; 95%Cl:29-96) of jackals trapped between 3-18 months in central South Africa. Larger studies with wider distribution of bait are needed to access the impact of oral rabies bait on rabies in jackals.

5.2 Introduction

Rabies is an important zoonotic disease caused by a rhabdovirus belonging to the *Lyssavirus* genus. It is a single-stranded, negative-sense RNA virus causing fatal encephalitis of domestic animals, humans and wildlife (Swanepoel et al., 1993). In most parts of Africa rabies is associated with the domestic dog and wildlife have not been considered important vectors (Wandeler, 1993) until more recently when it was established that jackal can sustain rabies infection without outside introductions from domestic dogs (Zulu et al., 2009). In southern Africa wildlife plays a significant role in the epidemiology of rabies. In Zambia and Namibia the black-backed jackal (*Canis mesomelas*) is the second most important host in the epidemiology of rabies after the domestic dog (Courtin et al., 2000; Röttcher and Sawchuk, 1978). In Zimbabwe the side-striped jackal (*Canis adustus*) and black-backed jackal are associated with 25% of rabies cases (Bingham et al., 1999). In South Africa, rabies is endemic due to its presence in domestic dogs as well as wildlife species such as black-backed jackal, yellow mongoose (*Cynictis penicillata*), and bat-eared foxes (*Otocyon megalotis*) (Swanepoel, 2004). Jackal rabies in South Africa accounts for 16% of all wildlife rabies cases (Chapter 3).

The first reported case of rabies in South Africa was in the Cape Province in 1882 from an infected dog that was brought in from England; it took 2 years to control the outbreak in dogs (Swanepoel et al., 1993). Wildlife played a minor role in rabies epidemiology until it was noted in the black-back jackal population in the 1950's in northern South Africa (Mansvelt, 1956). It continued to spread southwards and westwards in South Africa (Brückner et al., 1978). From there rabies became established in the bat-eared fox population in the western part of South Africa (Thomson and Meredith, 1993). The rabies virus in mongoose is genetically distinct from the canid biotype, and has been present in South Africa since the early 1800s (Van Zyl et al., 2010).

The effect of rabies on a naïve wild carnivore population can be profound. A 78% mortality was reported in the endangered Ethiopian wolf (*Canis simensis*) in an outbreak in the Ethiopian highlands in 2004 (Randall et al., 2004). During a rabies outbreak in the north western part of South Africa between 2013 and 2015, 23% of jackal territories were lost (Sabeta et al., 2018). The 2016 rabies outbreak in northern South Africa resulted in 76% of stable jackal territories being lost, and 93% of individual jackals (26/28) being lost in a monitored black-backed jackal population in the Broederstroom area, North West Province (Snyman, 2020).

Rabies in jackal in South Africa was controlled mainly by placement of poison bait and over 3,900 jackal were destroyed between 1951 and 1956 in the north-western parts of the country, keeping the jackal population below 0.6 per km^2 (Mansvelt, 1956). Poisoning was only partially effective as disturbance in the jackal population resulting in influx of jackal from surrounding areas. Rabies control by poisoning was continued until the

1980's (Bishop et al., 2010). Parenteral vaccination of livestock was started in 1977 in cattle in endemic areas with greater effect (Brückner et al., 1978).

Oral rabies vaccines in wildlife were first tested in the field in Switzerland in 1978 (Steck et al., 1982; Wandeler, 1988). Initially the Street Alabama Dufferin (SAD) strain was used but a total of ten different vaccines were used in Europe to control rabies in red foxes (*Vulpes vulpes*) over 30 years (Müller et al., 2015). The Raboral V-RG® (Merial, USA, now part of Boehringer Ingelheim) vaccine was one of these vaccines and was used in parts of France, Belgium and the Ukraine as part of their oral rabies vaccination strategies in wildlife between 1988 and 1993 (Müller et al., 2015).

The SAD (Berne) vaccine has been used in side-striped and black-backed jackal. All jackals had adequate antibody titres for 12 months post oral vaccination and survived challenge with a field strain of the rabies virus (Bingham et al., 1995). However, the SAD (Berne) vaccine has been shown to be pathogenic in a variety of rodent species as well as chacma baboons (*Papio ursinus*) (Bingham et al., 1992). It was therefore not suitable for southern Africa, where chacma baboons are common (Bingham et al., 1992). The SAG-2 (SAD Avirulent Gif) vaccine showed adequate protection in side-striped jackal, black-backed jackal and Ethiopian wolves (Sillero-Zubiri et al., 2016). Titres in side-striped jackal and black-backed jackal were adequate for 12 months post-consumption (Bingham et al., 1999b). Titres >0.5 IU/ml are recommended for dogs to be adequate to prevent rabies infection, while titres >0.25 IU/ml are thought to be adequate in foxes (*Vulpes* spp), raccoons (*Procyon lotor*) and raccoon dogs (*Nytereutes procyonoides*) (Moore et al., 2017; Office International Des Epizooties, 2008). There is no single cut-off level of rabies antibody titre that is invariable protective as small number of animals succumb to infection despite adequate antibody levels and some animals with no antibody titres after vaccination survive infection (Moore et al., 2017; Moore and Hanlon, 2010).

The antibody response to SAG-2 and V-RG® is similar in red foxes (Lambot et al., 2001). The V-RG® vaccine is a recombinant vaccinia virus vector vaccine expressing the rabies virus glycoprotein gene. It has been successfully used in a variety of wild carnivores such as red foxes in Europe, striped skunks (*Mephitis mephitis*), raccoons, artic foxes (*Vulpes lagopus*) and grey foxes (*Urocyon cinereoargenteus*) in the USA, and red foxes and golden jackal (*Canis aureus*) in Israel (Maki et al., 2017; Müller et al., 2015; Yakobson et al., 2006). The advantage of the V-RG® vaccine is its safety, with no risk of reverting to virulence in non-target species. A further advantage of the VR-G® vaccine is its stability in the environment with up to 3 weeks stability at an environmental temperature between 8 and 37°C (Maki et al., 2017) compared to the SAD vaccine which deteriorated in temperatures above 30°C (Mähl et al., 2014).

Antibody titres and duration of immunity for the V-RG® vaccine have not been established in black-backed jackals to date. The aim of this study was to investigate the duration of immunity in captive black-backed jackal over a 12-month period. In free-ranging jackals the percentage of animals with measurable antibody titres (≥0.2 IU/ml) was recorded after single placements of oral V-RG® vaccine in two protected areas.

5.3 Materials and Methods

This study was approved by the University of Pretoria Animal Ethics Committee (V149-16), the Department of Agriculture, Land Reform and Rural Development (DALRRD) (12/11/1/1/8) and South African National Parks (013-19).

Vaccine

Raboral V-RG® vaccine (Boehringer Ingelheim Animal Health, USA) containing 10^8 tissue culture infective dose 50% ($TCID_{50}$) per sachet (1.5 ml) (Maki et al., 2017) was used for the trial.

Captive jackals

The black-backed jackals were recruited opportunistically; any juvenile jackals admitted for rehabilitation in the northern parts of South Africa during 2018 were used, with the permission of the respective rehabilitation facilities. Eight animals were housed at a veterinary clinic in Mpumalanga Province, one at wildlife rehabilitation centre in Limpopo Province, and three at a wildlife rehabilitation centre and two at a zoological institution in Gauteng Province. All jackals were housed in groups. All jackals were between 3 to 6 months of age at the time of vaccination. Twelve black-backed jackals (8 males and 4 females) received oral rabies vaccine. Eight jackals were offered the vaccine in a sachet in food items (chicken pieces) and four jackals received the contents of the sachet drawn up into a 3 ml syringe and squirted directly into the mouth. Two jackals (1 male and 1 female) received a placebo containing a sachet of sterile water in a food item. The jackals were sampled prior to bait administration and at 4, 12, 24, 36 and 48 weeks afterwards if still in captivity. All jackals were caught with nets and hand restrained for blood collection from the cephalic vein into a 4 ml serum tube. No sedation was used in captive jackals for blood collection. These twelve jackals were closely monitored for the first 12 weeks after vaccination for any negative side-effects. Nine jackals were sampled at four weeks, ten at 12 weeks, six jackals at 24 weeks and three jackals at 36 and 48 weeks. All jackals were released post rehabilitation back into the wild at between 8 months and 15 months of age in game reserves in Mpumalanga, Limpopo and Gauteng.

Free-ranging jackals

Three sites in Gauteng Province, northern South Africa (Figure 5.1), and one site in the central plateau of the Free State Province (Figure 5.2), were selected for bait placements, due to ongoing jackal research at these sites. It was aimed to sample 25 jackals in each study area. However, since resources were limited and capture opportunities were determined by other research activities, sampling was opportunistic".

Study area 1

The three sites in northern South Africa were in protected areas in the Cradle of Humankind, a UNESCO World Heritage Site, within 50 km of the metropolitan areas of Johannesburg and Pretoria. The sites were i) the John Nash and Malapa Reserve (67.3 km^2), which is situated in the centre of the Cradle; ii) the Cradle Nature Reserve (16 km^2), situated to the east of the John Nash and Malapa Reserve; and iii) the Lion and Safari Park (17.7 km^2), to the north-east of the Cradle. The areas are all classified as rocky Highveld grassland (Kuhn, 2014) (Figure 5.1).

There were 40 jackals at the John Nash and Malapa Reserve during the 2016 census. Despite game fencing between the John Nash and Malapa Reserve and Cradle Nature Reserve, jackal cross between the reserves freely. The jackal density is estimated to be the same at the Cradle Reserve (0.6 jackal/km^2). The jackal density at Lion and Safari Park was estimated at 0.64 jackal/km^2 (Snyman, 2020).

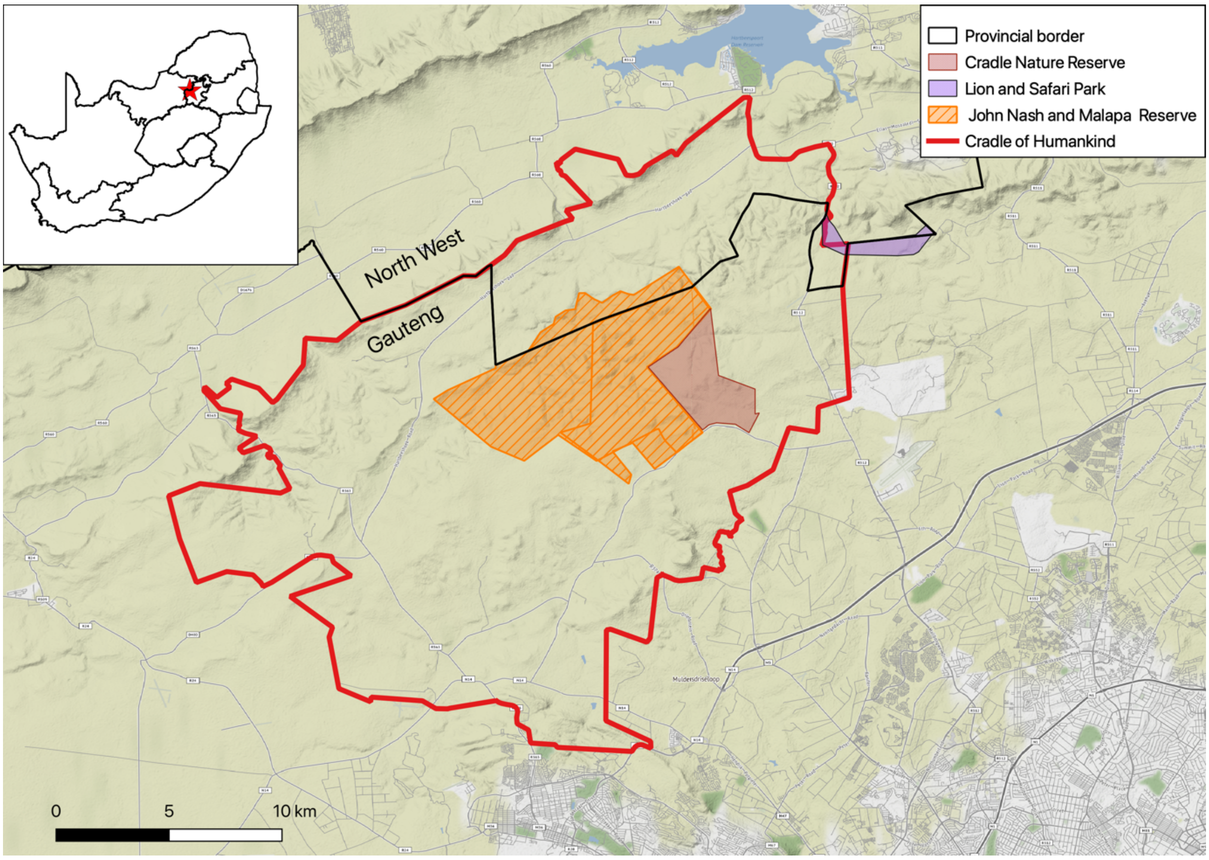

Figure 5.1: Map showing the Cradle of Humankind in Gauteng and North West Province, South Africa, with the John Nash and Malapa Reserve, Cradle Nature Reserve and the Lion and Safari Park. (The map was constructed using QGIS 3.4 using country boundaries and protected area boundaries downloaded and used with permission from protectedplanet.net.)

At the three Gauteng sites the bait was presented either in chicken heads (n=270) or fishmeal polymer (n=179), as these are preferred by jackal (Koeppel et al., 2020; Chapter 4). The baits in John Nash and Malapa Reserve (chicken head n=206, fishmeal polymer n=150) were distributed on three dates, approximately 2-3 weeks apart between September and October 2017, while the Cradle Nature Reserve was baited once (chicken head n=20, fishmeal polymer n=15). The baits (chicken head n= 44, fishmeal polymer n=14) at the Lion and Safari Park were distributed on two dates, 4 weeks apart in November 2017. The baits were distributed from vehicles, or on foot near jackal dens where access was limited. Baiting density varied according to road availability in the reserves. Jackals were subsequently trapped in all areas, beginning 3 months after bait placement and for up to 23 months.

Study area 2

The Golden Gate National Park (GGNP), in the Free State Province, bordering the mountain kingdom of Lesotho, served as the fourth study site. The park covers an area of 340 km^2 and is made up of sandstone formations interspersed with rich Highveld and Montane grassland as well as some Afromontane forests.

Twenty-seven oral bait rabies vaccines were placed at five sites in the GGNP in July 2018 (Figure 5.2). The census for 2016, 2017 and 2018 showed 48, 73 and 39 jackals at GGNP estimating the average density of one jackal per 8.7 km^2 or 0.12 jackals/km^2 (Brüns A, unpublished data). There are no large urban areas near this site.

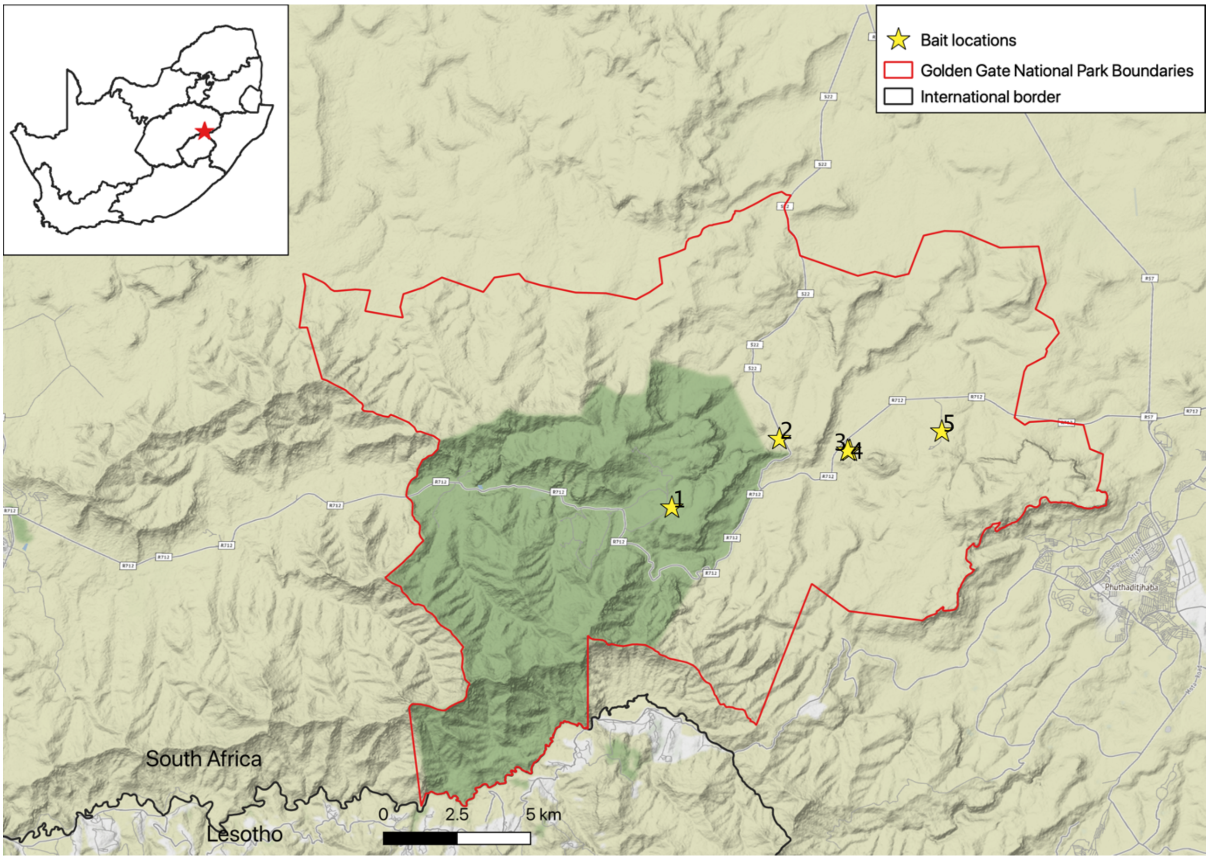

Figure 5.2: Oral rabies vaccine baiting locations (1-5) at Golden Gate National Park, Free State Province, South Africa. Highland grasslands are shown in dark green. (The map was constructed using QGIS 3.4 using country boundaries and GGNP boundaries with permission from South African National Parks.)

Due to the relative inaccessibility of the reserve, baits were placed along roads at monitored sites, with the exception of bait location 1, which was at a vulture restaurant. A total of 37 baits were placed: five baits per location per night. Only baits that were taken were replaced. Bait placement was 0.1 baits/km^2 at the GGNP (Table 5.1). Thirty-seven fishmeal polymer baits were placed at five sites at GGNP over two nights in July 2018. Jackals were trapped at 3 months and 18 months after baiting.

Between 3 and 23 months after bait placements jackals were captured by trapping or chemical immobilisation, for blood sample collection. They were either darted from a vehicle using a X-caliber dart CO_2 gun (Pneu-Dart INC, Williamssport, Pennsylvania, USA) with a 0.5 cc dart with barbed 3/8 inch needle (Motsumi Dart, Pretoria, South Africa) or caught using a Soft catch trap Fox (Oneida Victor® Animal Trap, Cleveland, Ohio, USA). Traps were buried 15 to 20 cm deep and covered with loose soil (Kamler et al., 2008). Traps were set at 50 N (50% of adult jackal weight) to prevent accidental capture of smaller species (Wildlife Control Supplies, 2020). Traps were set at sunset and checked every 2 hours throughout the night. Jackals were restrained with a net and hand injected intramuscularly with either a combination of metonil (medetomidine, Wildlife Pharmaceuticals (Pty) Ltd, Whiteriver, SA) 0.03-0.05 mg/kg and zoletil (zolazepam and tiletamine, Pfizer Laboratories (Pty) Ltd, Johannesburg, SA) 3-4 mg/kg or Bamanil (butorphanol 30mg/ml, azaperone 12 mg/ml, medetomidine 12mg/ml; Wildlife Pharmaceuticals (Pty) Ltd, Whiteriver, SA) 0.01-0.06 ml/kg. The same drug combinations were used for darting.

Jackals were aged by body size and teeth wear (Lombaard, 1971). Juveniles were classified as less than seven months old based on their deciduous teeth. Jackals between seven months to 2 years were identified as sub-adults by permanent canines and body size. Jackals greater than 2 years of age, according to teeth wear and body size, were classified as adults (Bingham and Purchase, 2003). All jackals were ear notched for identification.

Sample processing

Blood samples were refrigerated (4°C) shortly after collection until processing. Blood tubes were centrifuged and serum was separated and stored at -80°C prior to analysis at

ARC-Onderstepoort Veterinary Institute, South Africa. Titres were determined using the World Organisation for Animal Health (OIE) fluorescent antibody virus neutralisation test and was calculated against OIE dog reference serum. Neutralising antibody titres >0.5 IU/ml were considered to be adequate according to the World Health Organisation (WHO) and OIE guidelines (Office International Des Epizooties, 2008). Antibody titres of between 0.2 and 0.5 IU/ml were considered weak positive.

Statistical analysis

Stata 14 (StataCorp, College Station, TX, U.S.A.) was used for statistical analysis; significance was assessed at $p<0.05$. The Kruskal-Wallis rank sum test was used to compare rabies antibody titres between areas. Box and whisker plots were generated displaying the interquartile range within the box, with the whiskers indicating the 95% range. Maps were generated using QGIS (Version 3.4.9).

5.4 Results

Captive jackals

No side-effects were noted in any of the vaccinated jackals. No jackals had positive antibody titres against rabies prior to vaccination (median 0.1 IU/ml, IQR: 0-0.2) (Office International Des Epizooties, 2008). Ten of the 12 jackals that received oral rabies vaccine seroconverted after oral rabies vaccination, resulting in an estimated seroconversion proportion of 83% (95%CI: 0.52-0.98). The median titre was 2.05 IU/ml (IQR: 0.6-5.7) at 4 weeks post vaccination (n= 9) (Figure 5.3). One of the jackals could not be sampled at four weeks post vaccination. The median titre at 12 weeks was 3.5 IU/ml (n=10; IQR: 1.5-8.3). At 24 weeks the median was 2.9 IU/ml (n= 6; IQR: 0.8-72.8), at 36 weeks the median was 1.1 IU/ml (n=3; IQR: 0.9-2.9), and at 48 weeks the median was 3.4 IU/ml (n=3; IQR: 2-6.25) (Table 5.1). The two jackals which received placebo vaccine did not seroconvert.

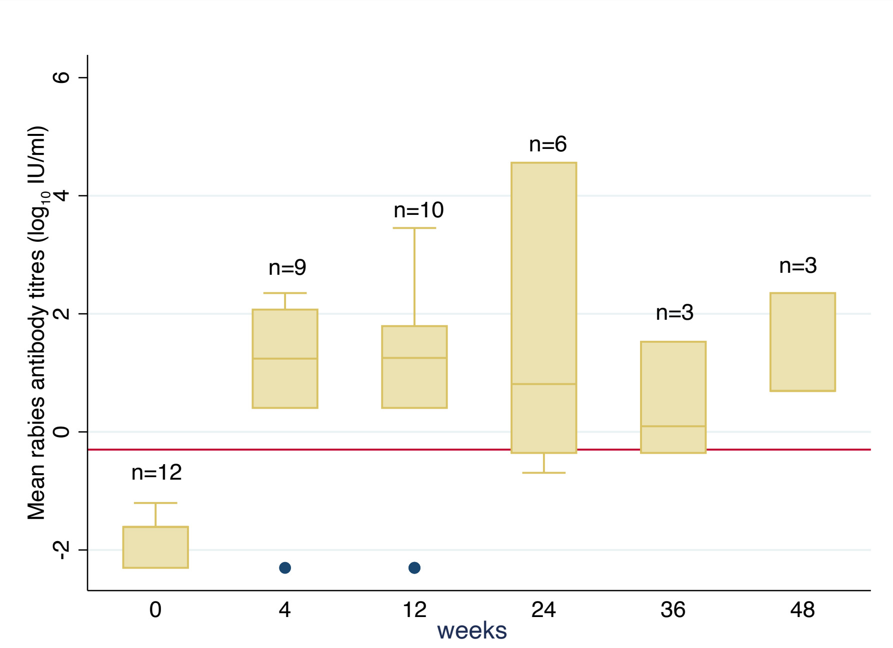

Figure 5.3: Rabies antibody titres in captive black-backed jackal (n=12) given oral rabies vaccine. Red reference line =adequate antibody titre (0.5 IU/ml).

Table 5.1: Serum neutralizing antibody level (IU/ml) in captive black-backed jackal given Raboral V-RG® vaccine for 48 weeks post vaccination

Jackal ID	Sex	Intervals after vaccination (weeks)					
		0	4	12	24	36	48
#22557	male	0.2	0	0.1			
#68887	male	0	0.1	0.1			
#24360	male	0.1	6	10.5			
#32910	male	0.2	10.5	3.5			
#32888	male	0.1		31.6			
#22689	female	0	10.5	4.6			
Eddie	male	0	2.6	3.5	4.6		
#68887	male	0.1	4.6	6	1.1	4.6	2
#68886	male	0.2	1.5	1.5	0.7	0.7	10.5
#68889	female	0.2	1.5	1.5	0.5	1.1	2
#65930	female	0			95.5		
#65986	female	0			95.5		

Antibody titres were significantly higher at 4, 12 and 24 weeks ($p = 0.037$) in jackal when the vaccine was administered directly into the mouth (n=4) compared to presentation of vaccine in food items (n=8) (Figure 5.4).

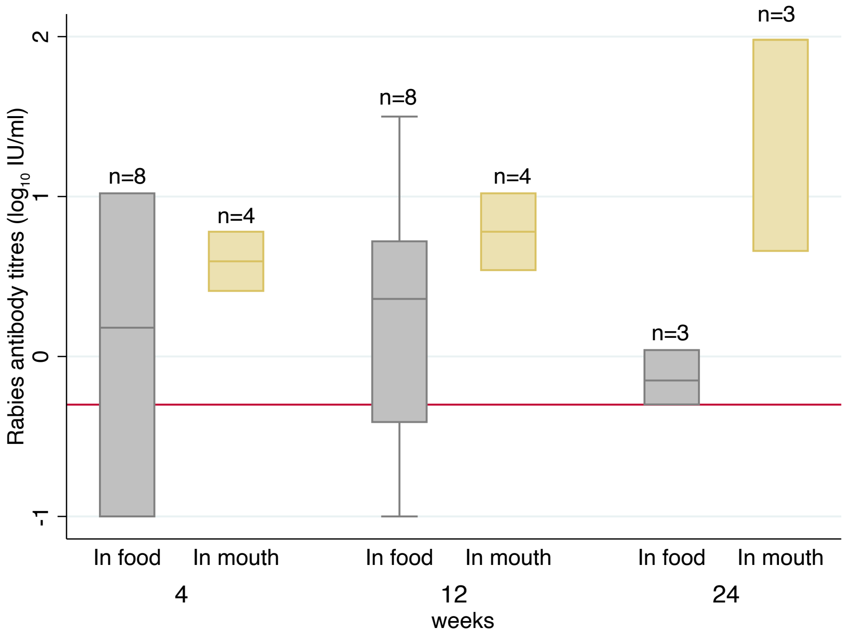

Figure 5.4: Comparison of rabies antibody titres after administration of rabies vaccine in food versus orally in captive black-backed jackals at 4, 12 and 24 weeks. Red reference line = adequate antibody titre (0.5 IU/ml).

Free-ranging jackals

Baiting density varied; the John Nash and Malapa Reserve was baited with 5.3 baits/km^2, the Cradle Nature Reserve received 2.2 baits/km^2 and the Lion and Safari Park was baited with 3.3 baits/km^2 (Table 5.4).

In the northern part of South Africa (Gauteng reserves), six jackals had been sampled to assess rabies antibody titre in 2014 and 2015 at the Lion and Safari Park and all were negative (n=6; 54%; 95%CI:0.23-0.83) (Snyman, 2020). Eleven jackals (4 males; 7 females) were caught between three and 23 months after baiting at the three Gauteng sites. One was a juvenile, two were sub-adults and eight were mature adults.

Four of the eleven jackals (36%; 95%CI:0.11-0.69) had antibody titres against rabies virus (Table 5.2). One jackal had adequate levels at 44 weeks after vaccination and three jackals had low rabies antibody titres at 12, 84 and 88 weeks.

Table 5.2: Rabies antibody titre (IU/ml) of 22 free ranging black-backed jackals in two areas (Gauteng and Free State Province) of South Africa after vaccination with Raboral V-RG® vaccine. Sero-positive titres above 0.5 IU/ml are marked with an asterisk (World Health Organisation, 2007).

Jackal ID	Area	Sex	Age Class	Intervals after vaccination (weeks)							
				0	12	44	48	72	84	88	92
LSP1	Gauteng Reserves	M	Juvenile		0.2						
JN2	Gauteng Reserves	F	Sub-adult			0					
JN3	Gauteng Reserves	F	Adult			3.5*					
JN4	Gauteng Reserves	F	Adult			0					
JN5	Gauteng Reserves	F	Adult				0.1				
JN6	Gauteng Reserves	M	Adult						0.4		
CR3	Gauteng Reserves	F	Adult							0.4	
JN7	Gauteng Reserves	F	Adult							0.1	
JN8	Gauteng Reserves	F	Adult							0	
JN13	Gauteng Reserves	M	Adult								0.1
JN14	Gauteng Reserves	M	Sub-adult								0
GG1	Free State Reserve	M	Adult	0.1							
GG2	Free State Reserve	F	Sub-adult	0.1							
GG3	Free State Reserve	M	Sub-adult	0.1							
GG4	Free State Reserve	F	Sub-adult	0.1							
GG5	Free State Reserve	M	Sub-adult		0.9*						
GG6	Free State Reserve	F	Adult		0.1						
GG7	Free State Reserve	F	Sub-adult		0.1						
GG8	Free State Reserve	F	Adult		1.5*						
GG11	Free State Reserve	F	Juvenile					0.4			
GG12	Free State Reserve	F	Juvenile					0.2			
GG13	Free State Reserve	F	Juvenile					1.1*			

At GGNP (Free State province) four jackals (2 males; 2 females;~10% of population) were caught prior to bait placement; none had detectable rabies antibody titres (Table 5.2). There was a low uptake of baits at certain sites with no baits taken at Basotho village (No 5) and Mooihoek (No2) as there was no jackal activity in those areas. All baits were taken at the vulture restaurant (No1) and Cilasberg West (No 3), and 40% of bait was consumed at Cilasberg East (No 4) (Table 5.3, Figure 5.2).

Table 5.3: Bait uptake by jackal at five locations at Golden Gate National Park, South Africa

Bait Location	Total No of baits placed	Total No of baits taken	Percentage taken
Basothu Village	5	0	0
Cilasberg4	7	7	100
Cilasberg2R	10	10	100
Mooihoek	5	0	0
Vulture restaurant	10	10	100
Total	37	27	73

Three months after placement of rabies bait, four jackals were captured at two sites at the vulture restaurant (No 1) and Cilasberg West and East (No 3&4). Three jackals were captured 18 months after bait placement at vulture restaurant. Three jackals were males and eight females. No jackal was captured more than once, as individual were identified via ear notching. Of the seven jackals (1 male, 6 females) captured after baiting, five (71%; 95%CI:0.29-0.96) had detectable rabies antibody titres, three of which(42%; 95%CI:0.1-0.8) were >0,5 IU/ml (Table 5.2). Thee jackals captured at 18 months post vaccination were juveniles estimated at below 14 weeks of age. There was no significant difference in titres between the jackals from the two provinces (p=0.108) (Table 5.4).

Table 5.4: Jackal population per km^2, bait density, number of jackal sampled after vaccination (n) and number of rabies antibody (Ab) positive jackals and percentage of positive jackals in three Gauteng and one Free State Reserves from 2018 to 2020, South Africa.

Reserve	Jackal/km^2	baits/km^2	Jackal tested (n)	Ab Positive jackals	% positive
John Nash and Malapa Reserve (GP)	0.6	5.3	9	2	22
Cradle Nature Reserve (GP)	0.6	2.2	1	1	100
Lion and Safari Park (GP)	0.64	3.3	1	1	100
Golden Gate National Park (FS)	0.12	0.1	7	5	71

5.5 Discussion

This study has shown that the Raboral V-RG® oral rabies vaccine results in adequate antibody titres for up to 12 months after vaccination in captive black-backed jackals. The titres in the captive jackals indicated most likely protective immunity from 4 to 48 weeks after initial vaccination without a booster vaccination. Only a single bait was given in this study as recommended by manufacture. Oral booster vaccination with either SAG2 or V-RG® within 1 month of initial vaccination did not improve antibody titres in foxes (Lambot et al., 2001) and black-backed jackal had adequate antibody titres for up to 12 months after single oral SAG2 bait vaccine (Bingham et al., 1999b). This is in contrast to wild dogs (*Lycaon pictus*) in which neutralising antibodies are short-lived without a booster vaccination with both oral and parenteral vaccines (Hofmeyr et al., 2000).

Much higher virus titres are required by striped skunks to produce an adequate immune response compared to red foxes (Vos et al., 2017). Effects of vaccine dose on titres was not tested in this study in a controlled environment as each jackal only received one dose of vaccine. The type of recombinant vaccine used can also affect the immune response A higher percentages of antibody positive raccoons have been noted with the use of ONRAB (Artemis Technologies, Guelph, Canada) compared to VR-G® vaccine with similar baiting densities (Mainguy et al., 2013, 2012), showing the importance of selecting the best vaccine for the species involved. Golden jackal however did show adequate antibody response to the V-RG® vaccine in a controlled environment with 78% of vaccinated jackal surviving challenge with RABV making it a suitable vaccine to be used in this study as golden and black backed jackals are closely related (Yakobson et al., 2006).

Two jackals did not seroconvert at either 4 or 12 weeks in this study. It is likely that these two swallowed the food item whole without penetrating the sachet and the vaccine would have been rapidly degraded in the gastrointestinal tract (Vos et al., 2017). Mean titres were significantly higher in jackal that received the vaccine squirted directly into mouth versus in food items. This is most likely due to higher doses of attenuated virus being present when squirted directly into the mouth compared to being taken in food items (Bingham et al., 1995; Grosenbaugh et al., 2007; Wandeler, 1988).

A combined sample size of ten jackals all tested negative between the two areas of South Africa prior to bait placement. The sample size was too small to rule out any antibody levels in the population. Jackal can survive natural rabies infection, although this is very rare. Antibody levels were found in 3% of jackals in Zimbabwe (5/156) and Kenya (1/28)

(Alexander et al., 1994; Foggin, 1988). Bellan et al., (2012) reported 9% of free-ranging jackals (n=81) to have antibodies to rabies in Etosha National Park, Namibia, but some of those jackals were incubating rabies at time of sampling. Presence of antibodies in a population has been linked to the sampling period with antibodies being more likely present during rabies epidemics (Bingham and Foggin, 1993).

After oral bait rabies vaccination 36% of jackals trapped in Gauteng had rabies antibody titres, resulting from an average baiting density of 3.6 baits/km^2. This was similar to the 29.5% seropositive samples reported in golden jackal and red foxes in Israel resulting from a baiting density of 14 to 19 baits/km^2 (Yakobson et al., 2006). It is possible that some of the titres were due to previous exposure to rabies but there were no recorded outbreaks in the area in 2017 to 2019 making it unlikely to be associated with exposure to RABV (Chapter 3). The percentage of jackal having antibody titres at GGNP was higher, with 71% of jackals captured having titres present. In raccoons the percentage of animals with rabies antibody titres after oral bait vaccination was related to number of baits in an area, with more baits per km^2 resulting in a higher proportion of animals with antibody titres (Rosatte et al., 2008). Bait density was low in both area but bait placement by hand and from vehicles has been shown to target more suitable habitat and den sites, making it more efficient than distribution by aircraft. In raccoons hand baiting was more effective then helicopter distribution, especially in urban areas (Boulanger et al., 2008).

In Gauteng jackals were captured for up to 92 weeks after placement of rabies bait, which might have resulted in lower or no titres as rabies titres decrease with time in comparison to GGNP were jackal were only tested up to 18 months post bait placement. The titres in captive jackals in this study remained adequate for 48 weeks which is similar to foxes vaccinated with recombinant vaccine which lasted for 48 weeks (Brochier et al., 1988) and racoons which had titres for up to 56 weeks (Brown et al., 2011). Captive golden jackal had a sero-conversion of 44% after oral bait consumptiona and 78% survival to challenge with field strain (Yakobson et al., 2006).

There was an estimated population of 0.64 jackal per km^2 at the eastern side of the Cradle of humankind (Snyman, 2020). This is similar to the estimated population of 0.6 jackal per km^2 at the John Nash and Cradle Reserves (Hennie Visser, personal communication).

This is in contrast to GGNP where there was 0.12 jackal per km^2, only one sixth the population density of the Cradle. Pup survival was estimated to be about 55-60% in the Gauteng area (Snyman, 2020), slightly lower than the 66% reported in the montane region of the Drakensberg (Rowe-Rowe, 1982). There was also a difference in bait type used with both chicken heads and fishmeal polymer used in northern South Africa and only fishmeal in central area. It is unlikely that the bait type used had a great effect on uptake at the central area as there is low density of non-target species in the area and therefore competition for bait is reduced and jackal consumed both equally (Chapter 4)

The higher percentage of sero-positive jackals at GGNP might be associated with a reduced population turn over but further studies are needed to verify this. Higher population turnover will require higher density of bait placement or more frequent application to result in adequate antibody levels in the population (Holmala and Kauhala, 2006). It has been shown that rabies antibody titres can be transferred from vaccinated foxes to kits. The rabies antibody titre in the fox kits declined between 45 to 75 days after birth (Lambot et al., 2001). All three juvenile jackals at GGNP had rabies antibody titres present in our study, one above 0.5 IU/ml. They were all below 14 weeks of age according to their dentition (Lombaard, 1971); therefore, they could not have been exposed to original bait but might have received antibodies through milk.

Rabies in jackals poses a risk not only to livestock and domestic animals, but also to endangered wildlife species. Rabid jackals resulted in two rabies outbreaks which caused up to 87% mortality in the affected wild dog packs at the Madikwe Game Reserve in the northwest of South Africa (Hofmeyr et al., 2000, 2004). In foxes it has been suggested that a barrier of 60% immune animals will prevent the spread of rabies, while strategic placement of vaccine along natural barriers can be more efficient, requiring fewer animals to be vaccinated (Haydon et al., 2006; Wandeler et al., 1988). These could be applied to areas like the GGNP were jackals travel nearly exclusively through the valleys. Bait placement in this study was along the game paths and roads frequented by jackals only covering a small area of the park, yet a high proportion of jackals (71%) had titres after bait placement showing that natural barriers such as mountain ranges or river can aid in the distribution of baits.

Due to the low numbers of wild jackal included in this study, a high degree of uncertainty regarding the initial seroprevalence of rabies antibody titres in the study areas exists. Furthermore, the inability to follow up on individual animals also subjected our estimates to a large degree of uncertainty. However, the study in captive jackals showed that oral vaccination can result in adequate levels of long-lived antibody titres in vaccinated animals. More intensive studies increasing the area covered could yield more precise estimates of the level of baiting required in free-ranging jackal populations to ensure adequate population immunity to rabies virus. The optimal bait density might vary between regions and density of jackal populations. Ideally jackal carcasses in areas covered by vaccination should be evaluated for tetracycline biomarker presence (Robardet et al., 2012) and antibody titres (fresh carcass only) to determine the effect of bait placement in the areas and correlated with bait density and land type use.

5.6 Conclusion

Captive black-backed jackal vaccinated with Raboral V-RG® vaccine in oral bait resulted in adequate antibody titres against rabies for a period of up to 48 weeks in the majority of animals. Use of the Raboral V-RG® vaccine in free-ranging jackal in four protected areas resulted in rabies antibody titres detected in between 36% and 71% of the sampled population. This was likely influenced by, amongst other factors, baiting density, jackal density and population turnover. More research would be required to evaluate the impact of oral rabies vaccination on population immunity and the occurrence of rabies outbreaks in jackals.

CHAPTER 6

General Discussion

Rabies has been researched extensively in the last 55 years. The number of publications has increased from fewer than ten publications before 1970, to over 400 publications annually since 2011 (Jagannara, 2015). Over 900 scientific papers on rabies were published in 2020 alone (Google Scholar, October 2020). This heightened research interest has seen knowledge of rabies increasing, not only on a molecular level but also its epidemiology. Despite being considered a fatal disease once clinical signs are evident, it is completely preventable in humans after suspected exposure by the use of post-exposure prophylaxis. Yet 21 000 deaths in humans occur annually in Africa due to exposure to rabid dogs (Hampson et al., 2015; Mbilo et al., 2020). Reducing rabies results in fewer human deaths and reduces economic losses due to livestock mortality. To try to address these deaths, the World Health Organisation and other international agencies launched the Unite Against Rabies campaign to eliminate dog-mediated rabies by 2030 (United Against Rabies Collaboration, 2019).

The first reported case of rabies in South Africa was in a dog in 1882 (Swanepoel et al., 1993), and dog-mediated rabies remains an issue in the densely populated areas of KZN and EC (Coetzee and Nel, 2007). This is also reflected in the spatiotemporal analysis (Chapter 3), with the most statistically significant cluster (cluster 1) over the last 26 years in KZN. The KZN Province has the highest number of rabies cases in South Africa due to its dense population and high turnover of dogs, making it a challenge to address, especially in the context of eradicating canine-associated rabies by 2030 (Hergert et al., 2018; United Against Rabies Collaboration, 2019). The termination of cluster 1 by sustained and targeted dog vaccination (LeRoux et al., 2018) highlights the importance of understanding local hotspots and identifying clusters.

In Africa, multiple wildlife vectors are responsible for the transmission and maintenance of RABV. The highest percentage of rabies cases in wildlife has been reported in Zimbabwe (25%) with Namibia and South Africa (Chapter 3) having the next highest percentage (19%), followed by Botswana (15%) and Zambia (4.5%) (Bingham et al., 1999; Courtin et al., 2000; Masupu et al., 1999; Munang'andu et al., 2011).

South Africa has the widest range of host species of wildlife involved in rabies epidemics in southern Africa, with yellow mongoose, bat-eared fox, black-backed jackal, meerkat and aardwolf being the wildlife species mostly involved in the spread of rabies. Meerkats are involved due to their close association with the yellow mongoose, as they often share burrows and so increase the risk of rabies spread (Swanepoel et al., 1993). The yellow mongoose was the most important wildlife vector, followed by the bat-eared fox until 1993 (Swanepoel et al., 1993) After the yellow mongoose, the black-backed jackal and the bat-eared fox are currently the other two most important wildlife hosts in South Africa since 1993 (Chapter 3). The work presented here shows that wildlife played a significant part in the epidemiology of rabies in South Africa from 1993 to 2019, with 69% (95%CI: 0.39-0.90) of identified clusters having wildlife involvement. Wildlife rabies cases occurred throughout South Africa, although wildlife cases depended on species distribution and density of wildlife in the area, as well as land use. Reducing rabies in wildlife and dogs can assist with reduction of rabies in endangered species such as the wild dog. Oral rabies vaccination should be considered in areas with wildlife clusters especially when black-backed jackals are involved.

The black-backed jackal is one of the primary wildlife vectors in South Africa today, with the density of jackals critical in the epidemiology of rabies. Jackals accounted for 15.5% of wildlife rabies in this study, with an increase of 10.5% in jackal-associated rabies in recent years (1993 to 2019), compared to the 4.8% reported before 1993 (Swanepoel et al., 1993). Disturbance in wild jackal populations due to changes in farming practices and increases in urbanisation is likely to have increased jackal-associated rabies. Jackal increase their aggression and dispersal distance when there is an increase in vacant territories (Loveridge and Macdonald, 2001). Jackal rabies cases peaked from July to August, the drier colder months of the year (July to August) that correspond with the jackal mating season (Rowe-Rowe, 1975; Skinner and Chimimba, 2005b). Increased movement of jackals has been shown to correspond to the higher number of rabies cases in jackals (Chapter 3).

Black-backed jackals are found in the northern parts of South Africa in a variety of biomes (Skinner and Chimimba, 2005b). In the eastern parts of South Africa, the population density is around 0.6 jackal/km^2, which is likely to support epidemics once rabies has been introduced into the population: jackal densities of 1 to 1.4 jackal/km^2 can maintain rabies infection in a population without external introductions, as was seen in Zimbabwe (Rhodes et al., 1998). In the montane areas of the Drakensberg (KZN Province) the density of jackals has been recorded as 0.4 jackal/km^2 (Rowe-Rowe, 1982), compared to the 0.12 jackal/km^2 recorded in the nearby GGNP in this study. The densities in these montane areas may not be able to support sustained rabies transmission in jackal. Hence, the density of jackal should be taken into account when evaluating the need to control rabies in jackal populations, and it may not be necessary to include vaccination of jackals where their population density is low. However, more work needs to be done to determine the relationship between jackal population density and its effect on rabies vaccinations requirements. There was a small sample size in this study and bait uptake should be repeated over larger areas and various land-use types.

Rabies can be controlled by several methods, including i) culling or contraceptive use to reduce vector populations, ii) parenteral vaccination of dogs, selected wildlife and humans, and iii) oral bait vaccination of dogs and wildlife vectors. Culling, however, has negative impacts especially when poisoning is used, such as mortality in non-target species, including species that are threatened with extinction, and disturbance of mesopredator balance; (Linhart et al., 1993; Mansvelt, 1956). Reducing rabies in wildlife might also result in the reduction of persecution of mesopredators. While parenteral vaccines are effective for humans and dogs, they are not feasible for control of RABV in wildlife vectors. However, administration of vaccines using oral bait has been successfully applied in controlling RABV in red foxes in Europe (Cliquet and Aubert, 2004; Müller et al., 2015), red foxes and golden jackal in Israel (Yakobson et al., 2006) and grey foxes in North America (Maki et al., 2017).

To evaluate the possibility of using oral rabies bait in the field, the acceptance of different bait types by black-backed jackals in South Africa was investigated. Oral rabies baits were placed both in the winter months when food availability was sparse, and in the summer months, when there was greater food availability in the area. Bait uptake by target species is usually higher when food availability is lower (Maki et al., 2017), but this study showed higher competition for bait by non-target species, such as omnivores and herbivores, during the winter months. To ensure increased uptake by jackal, summer months might therefore be better suited, ideally in December or January before pup dispersal (Loveridge and Macdonald, 2001) to ensure that vaccine is presented to all age groups of jackals in areas where competition with non-target species is likely to be high (Chapter 4). Vaccinations ideally should be done twice a year to increase the percentage of jackals exposed to the vaccine (Taylor et al., 2020). Winter vaccination ideally would be timed around August to September, after the mating season and once pups are weaned, so that bait could be consumed by them to increase the number of vaccinated jackal in the population (Bruyère et al., 2000), even though it has been shown in foxes that specific targeting of young foxes before dispersal had little effect on the spread of rabies (Vos, 2003). Black-backed jackals consumed 20% of bait, with an overall acceptance of 87% across all species (Chapter 4). They took chicken heads and commercial fish polymer bait equally, although the competition by non-target species is significantly less if chicken heads are used. Therefore a combination of chicken head and fish polymer was used to distribute vaccines to free-ranging jackals (Chapter 5). Chicken heads are more time consuming to prepare and cannot be stored for prolonged periods of time making them less suitable for large bait distributions.

First, this study tested the recombinant vaccina virus-rabies glycoprotein V-RG® vaccine in captive jackals, which resulted in adequate antibody titres for up to 12 months after vaccination with a single bait vaccine although no challenge study was performed. Rabies antibody titres are used as a guideline of protection, with some wildlife species such as striped skunks shown to be refractory to some oral rabies vaccines, even when high viral titres were used (Vos et al., 2017).

The study then tested the vaccine in free-ranging jackals. Oral rabies bait was placed in the field in the winter months at four sites in South Africa and two different biomes; the Afromontane biome in central South Africa at GGNP and the rocky highveld grassland biome in northern South Africa in the Cradle of Humankind. It has been suggested that higher daytime temperature could reduce the uptake of baits in red foxes (Taylor et al., 2020), but this was not seen in jackals in this study with jackals consuming baits equally during summer and winter months despite significant variation in mean daily temperature varying from 15°C in winter to 22°C in summer. All baits were placed at dusk, to decrease competition with non-target species.

Despite rabies outbreaks occurring in black-backed jackals in South Africa every 3 to 6 years, no naturally occurring RABV antibody could be found in ten jackals tested before distribution of rabies bait in both areas. In Zimbabwe and Kenya, 3% of jackal populations had rabies antibody titres (Alexander et al., 1994; Foggin, 1988), with a sample size of 184 jackals. The time of sampling is critical for the presence of rabies antibody titres since titres are more likely to be present during epidemics (Bingham and Foggin, 1993). The jackals in this study in the northern parts of South Africa were sampled before the 2016 epidemic.

Oral bait rabies vaccination resulted in adequate antibody titres in 36% of jackals trapped in northern parts compared to 71% in the central areas, although the high degree of uncertainty around these estimates due to the small sample sizes precludes any meaningful comparison. Baiting density varied considerably between the sites, with more baits placed in areas with higher jackal presence thus providing approximately between one to five baits/jackal, which was higher than rabies bait used in Ethiopian wolves by targeted delivery (Sillero-Zubiri et al., 2016) but lower than what was used by aerial distribution in Eurasian golden jackal (Yakobson et al., 2006). Both areas were baited by hand and from vehicles to target more suitable habitats, therefore making it more efficient than distribution by aircraft as game path and den sites could be specifically targeted

Titres in free-ranging populations decrease dramatically with the cessation of baiting, and therefore continued baiting on an annual and biannual basis is required to maintain adequate rabies antibody titres within a population. Baiting can only be stopped if rabies is controlled in the area and there is a low risk of reintroduction from the periphery. Vaccination might need to be continued along corridors or borders to prevent reintroduction into an area from neighbouring areas. In foxes, it has been suggested that a barrier of 60% immune animals will prevent the spread of rabies, while the strategic placement of vaccine along natural barriers can be more efficient, requiring fewer animals to be vaccinated (Haydon et al., 2006; Wandeler et al., 1988). The sample size was small in this study and baiting was done only in one season, repeated baiting campaigns over several seasons are needed to evaluate the effects on rabies in free-ranging jackal in South Africa.

Conclusions and recommendations

1. The spatiotemporal distribution of rabies in South Africa indicated that rabies is maintained by both dogs and wildlife with 15.8% of cases being associated with wildlife. This should be considered when deciding on oral rabies vaccine strategies and strategies should be decided on a national level taking into account trends in rabies cases and species involvement in different areas in South Africa. Dog vaccination on its own might not be effective in certain areas due to the high involvement of wildlife.

2. Jackals will eat either commercial fishmeal polymer or chicken head bait, but chicken heads are less likely to be taken by non-target species, making them more selective for jackals.

3. Raboral V-RG® vaccine achieved adequate antibody titres in black-backed jackal in a captive environment.

4. Raboral V-RG® vaccine does show promising results in free-ranging jackal but more work is needed on required baiting densities and time of year for distribution for different areas of South Africa.

5. Wildlife rabies could potentially be reduced by the application of oral rabies vaccine over a wider area and several seasons, and by evaluating the rabies antibody titres in wildlife over time.

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APPEXURE A – Animal Ethics Certificates

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**Bait preference trial to evaluate best method of offering Raboral V-RG vaccine to free ranging jackal (*Canis mesomelas*) and retrospect analysis of rabies antibody titre**
PROJECT NUMBER	**V127-16**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr K Koeppel**

STUDENT NUMBER (where applicable)	
DISSERTATION/THESIS SUBMITTED FOR	**Academic**

ANIMAL SPECIES	**Black-backed jackal (*Canis mesomelas*)**	
NUMBER OF DAYS OF MONITORING	**3**	
Approval period to use animals for research/testing purposes		**October 2016 – October 2017**
SUPERVISOR	**Prof P Thompson**	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 31 October 2016
CHAIRMAN: UP Animal Ethics Committee	Signature

S4285-15

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**Evaluation of the efficacy of oral rabies vaccine Raboral V-RG in the control of rabies in Black-backed Jackals (Canis mesomelas)**
PROJECT NUMBER	**V149-16**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr. K Koeppel**

STUDENT NUMBER (where applicable)	--
DISSERTATION/THESIS SUBMITTED FOR	**Academic**

ANIMAL ANIMALS	**Black-backed Jackals (Canis mesomelas)**	
NUMBER OF ANIMALS	**150 + 14**	
Approval period to use animals for research/testing purposes		**November 2016-November 2017**
SUPERVISOR	**Prof. P Thompson**	

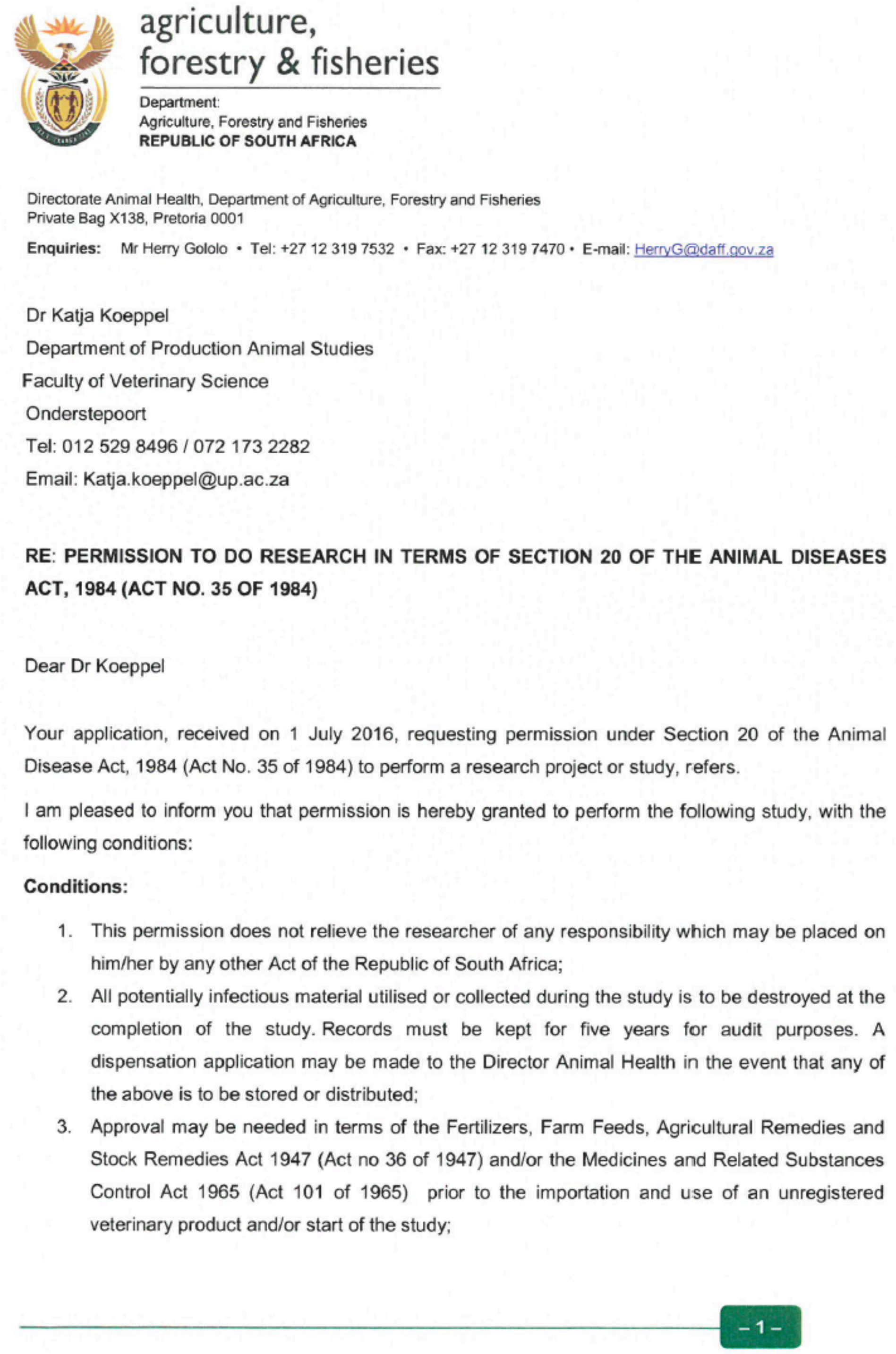
KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 28 November 2016
CHAIRMAN: UP Animal Ethics Committee	Signature

S4285-15

APPEXURE B –Section 20 approval



agriculture,
forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Directorate Animal Health, Department of Agriculture, Forestry and Fisheries
Private Bag X138, Pretoria 0001

Enquiries: Mr Herry Gololo • Tel: +27 12 319 7532 • Fax: +27 12 319 7470 • E-mail: HerryG@daff.gov.za

Dr Katja Koeppel
Department of Production Animal Studies
Faculty of Veterinary Science
Onderstepoort
Tel: 012 529 8496 / 072 173 2282
Email: Katja.koeppel@up.ac.za

RE: PERMISSION TO DO RESEARCH IN TERMS OF SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO. 35 OF 1984)

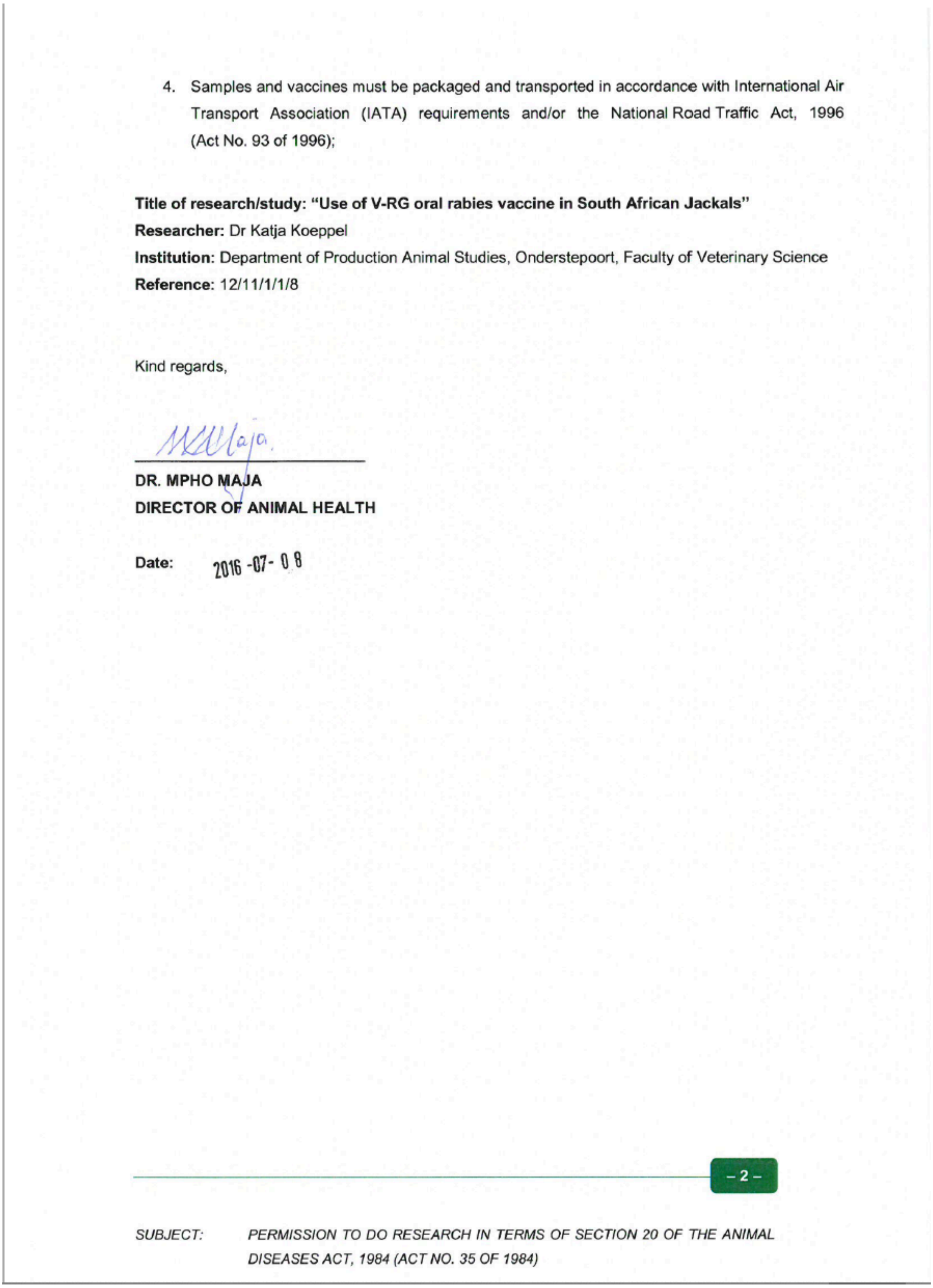
Dear Dr Koeppel

Your application, received on 1 July 2016, requesting permission under Section 20 of the Animal Disease Act, 1984 (Act No. 35 of 1984) to perform a research project or study, refers.

I am pleased to inform you that permission is hereby granted to perform the following study, with the following conditions:

Conditions:

1. This permission does not relieve the researcher of any responsibility which may be placed on him/her by any other Act of the Republic of South Africa;
2. All potentially infectious material utilised or collected during the study is to be destroyed at the completion of the study. Records must be kept for five years for audit purposes. A dispensation application may be made to the Director Animal Health in the event that any of the above is to be stored or distributed;
3. Approval may be needed in terms of the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act 1947 (Act no 36 of 1947) and/or the Medicines and Related Substances Control Act 1965 (Act 101 of 1965) prior to the importation and use of an unregistered veterinary product and/or start of the study;



4. Samples and vaccines must be packaged and transported in accordance with International Air Transport Association (IATA) requirements and/or the National Road Traffic Act, 1996 (Act No. 93 of 1996);

Title of research/study: "Use of V-RG oral rabies vaccine in South African Jackals"
Researcher: Dr Katja Koeppel
Institution: Department of Production Animal Studies, Onderstepoort, Faculty of Veterinary Science
Reference: 12/11/1/1/8

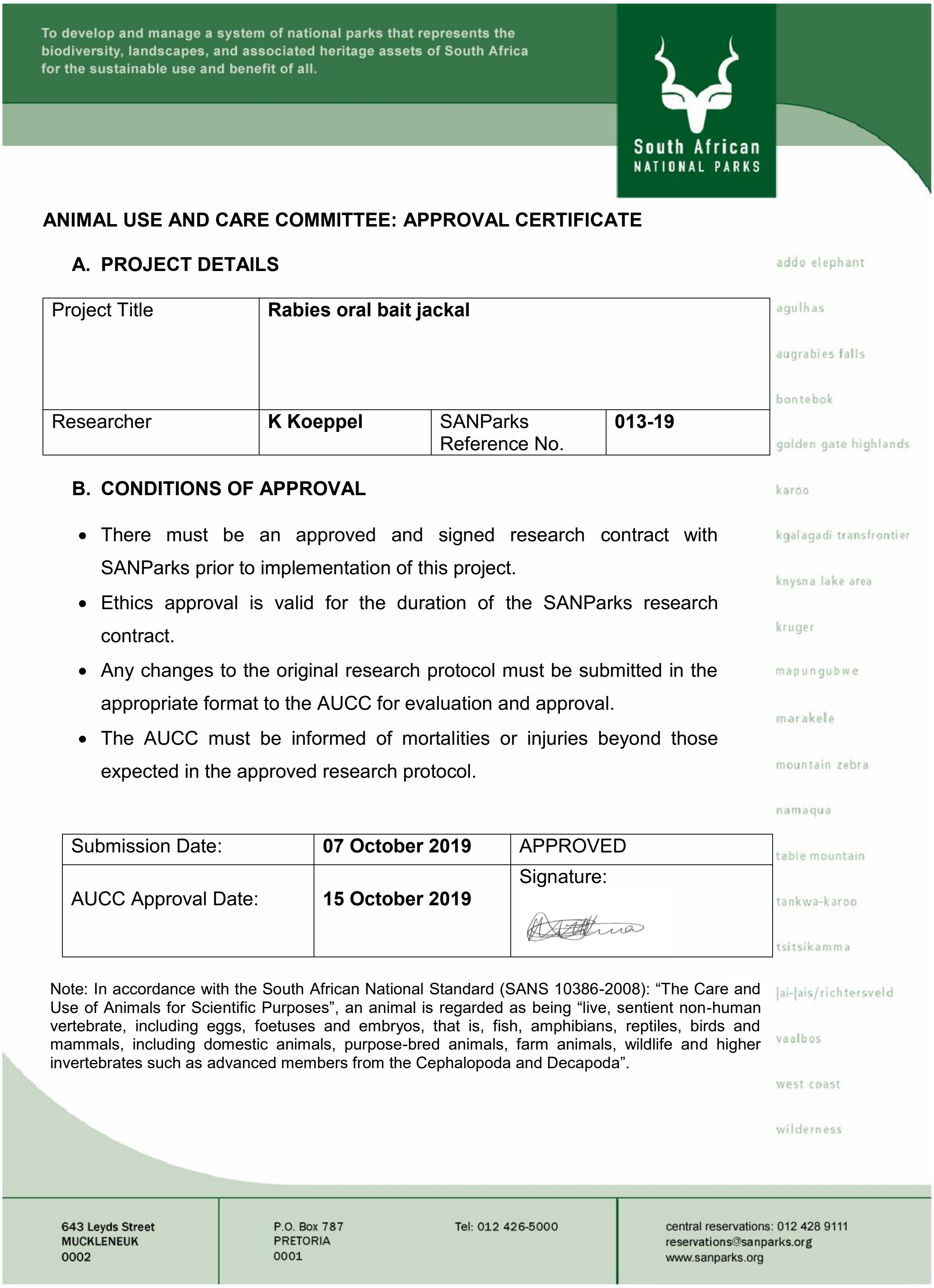
Kind regards,

MMaja.

DR. MPHO MAJA
DIRECTOR OF ANIMAL HEALTH

Date: 2016 -07- 08

APPEXURE C –SANParks Certificate



To develop and manage a system of national parks that represents the biodiversity, landscapes, and associated heritage assets of South Africa for the sustainable use and benefit of all.

South African
NATIONAL PARKS

ANIMAL USE AND CARE COMMITTEE: APPROVAL CERTIFICATE

A. PROJECT DETAILS

Project Title	**Rabies oral bait jackal**		
Researcher	**K Koeppel**	SANParks Reference No.	**013-19**

B. CONDITIONS OF APPROVAL

- There must be an approved and signed research contract with SANParks prior to implementation of this project.
- Ethics approval is valid for the duration of the SANParks research contract.
- Any changes to the original research protocol must be submitted in the appropriate format to the AUCC for evaluation and approval.
- The AUCC must be informed of mortalities or injuries beyond those expected in the approved research protocol.

Submission Date:	**07 October 2019**	APPROVED
AUCC Approval Date:	**15 October 2019**	Signature:

Note: In accordance with the South African National Standard (SANS 10386-2008): “The Care and Use of Animals for Scientific Purposes”, an animal is regarded as being “live, sentient non-human vertebrate, including eggs, foetuses and embryos, that is, fish, amphibians, reptiles, birds and mammals, including domestic animals, purpose-bred animals, farm animals, wildlife and higher invertebrates such as advanced members from the Cephalopoda and Decapoda”.

addo elephant
agulhas
augrabies falls
bontebok
golden gate highlands
karoo
kgalagadi transfrontier
knysna lake area
kruger
mapungubwe
marakele
mountain zebra
namaqua
table mountain
tankwa-karoo
tsitsikamma
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wilderness

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