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The Prevalence of Canine Distemper Virus Antibodies in Wild Carnivores in the Kruger National Park and Marakele National Park

BY

ANGÉLIQUE CRONWRIGHT-SNOEREN

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SUPERVISOR: **PROF MORITZ VAN VUUREN**

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DECLARATION

I declare that this dissertation hereby submitted to the University of Pretoria for the degree of Master of Science (Veterinary Tropical Diseases) has not been previously submitted by me for the degree at this or any other University, that it is my own work in design and in execution, and that all material contained therein has been duly acknowledged.

Signed :

Date :

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TABLE OF CONTENTS

TABLES

FIGURES

ABSTRACT

A description is given of a prevalence study of canine distemper virus antibodies in large carnivores in the Kruger National Park and Marakele National Park in South Africa. The serum-virus neutralization test was used to determine the prevalence of antibodies in different carnivore species in Kruger National Park and Marakele National Park. The species tested included 198 lions, 33 hyenas, 23 wild dogs and a leopard. The results demonstrated a total of 69 (34.8%) positive sera from lions. Moreover, CDV neutralizing antibodies were present in 25 (75.8%) hyena sera, 18 (78.3%) wild dog sera and the serum of one leopard. The results demonstrate that canine distemper virus is present in the Kruger National Park and that removal of the fences between the Kruger National Park and other conservation areas may pose a risk for spreading infectious diseases to susceptible species. Further work would be required to determine the prevalence of CDV in neighbouring regions. Only then risks can be estimated.

INTRODUCTION

Mozambique, South Africa and Zimbabwe signed an international treaty towards the establishment of the Greater Limpopo Transfrontier Park (GLTP) in 2000. The GLTP connects the Limpopo National Park (Mozambique), Kruger National Park (South Africa), Gonarezhou National Park, Manjinji Pan Sanctuary and Malipati Safari Area (Zimbabwe), the Sengwe communal land (Zimbabwe) and the Makuleke region (South Africa). The Limpopo Transfrontier park will have a total surface of approximately 35 000 km^2. The GLTP is the first phase of the total Greater Limpopo Transfrontier Conservation Area (GLTFCA) which will measure 99 800 km^2 (SANparks, 2009).

The GLTP will embrace the Kruger National Park which is presently one of the world's few conservation areas with significant and viable populations of wild dogs (*Lycaon pictus*) consisting of approximately 300 animals. Other significant populations of wild carnivores in the KNP include approximately 1 500 lions (*Panthere leo krugeri*), 2 000 spotted hyenas (*Crocuta crocuta)* and 1 000 leopards (*Panthera pardus pardus).*

The complete removal of the border fences between the areas will allow free movement of animals, which will lead to contiguous populations of animals. Opening of these areas may increase the risk for diseases to spread from these conservation areas to each other as all human habitation and domestic animals will not be removed out of the GLTFP. Emerging infectious diseases of wildlife play a substantial role in the health of endangered species. In contrast removal of the fences between the KNP, Mozambique and Zimbabwe, may contribute to the conservation of threatened animals such as the wild dog.

Opening up the conservation areas will be of benefit for the the Kruger National Park as the KNP has a surplus of elephants that through their behaviour destroy the environment. Culling elephants has always been a controversial issue and transferring the elephants to other National Parks was not always possible due to

financial or other implications. By opening up the conservation areas it can be assumed that the elephants will be able to roam free into three different regions of the GLTFCA and follow the natural migration routes. A further assumption is that the existing (elephant) populations will also be able to exchange genes with other elephants.

In terms of the geographic position of the GLTFCA, the main diseases of concern include bovine tuberculosis, bovine brucellosis, foot-and-mouth disease, theileriosis, canine distemper, rabies, malaria, anthrax, trypanosomosis, Rift Valley fever and African swine fever. Most of these diseases can be transmitted by wildlife or domestic animals. These diseases, except for malaria, are either carried by wildlife or can cause clinical disease in wildlife.

In this study sera from large wild carnivores from Marakele National Park were included although the Park is situated in the Northwest Province.

Clinical canine distemper has not previously been documented in the Kruger National Park (KNP) nor the Marakele National Park (MNP) (R. Bengis, state veterinarian, personal communication). Therefore the hypothesis of this study was stated as: “Clinical canine distemper is not widespread in wild carnivores in the Kruger National Park and Marakele National Park and will not represent a risk to carnivores in the GLTFCA when free movement of animals takes place”.

The main objective of this study was to; “Do a serological survey on sera from wild carnivores in the Kruger National Park and Marakele National Park and determine the prevalence of canine distemper virus (CDV) antibodies”. The aim of this study was to provide information to risk managers dealing with risk assessment of the establishment of the transfrontier conservation areas in southern Africa, with emphasis on the Greater Limpopo Transfrontier Park (GLTP). The GLTP is a collaborative project between different African countries to connect their conservation areas with each other including the Kruger National Park. Removing the fences between these areas to achieve that objective, will consequently lead to animals roaming free between the areas forming contiguous populations. This may represent a risk for transmission of diseases to susceptible animals.

LITERATURE REVIEW

1. The role of canine distemper virus in wildlife globally

Canine distemper is a contagious disease which occurs worldwide and affects domestic dogs as well as other carnivores. The status of canine distemper is of importance, when creating or merging new conservation areas.

1.1 Classification and characteristics of canine distemper virus

Canine distemper virus is a member of the *Morbillivirus* genus within the *Paramyxoviridae* family. There are several members of the *Morbillivirus* genus which include: measles virus, rinderpest virus, peste des petits ruminants virus, canine distemper virus, phocine distemper virus, porpoise distemper virus and dolphin distemper virus (Appel & Summers, 1995; Osterhaus, De Swart,Vos,Ross, Kenter & Barret, 1995).

1.2 Clinical signs of canine distemper

The clinical signs of canine distemper in both domestic and wild carnivores include fever, serous nasal disarges that rapidly become mucopurulent, oculonasal discharges, anorexia, diarrhoea, pneumonia, hyperkeratosis of the foot pads and epithelium of the nasal plane, gastro-intestinal lesions, neurological signs and death. Neurological signs include localised twitching, tremors, disorientation, paresis or paralysis beginning in the hindlimbs (ataxia), epileptiform convulsions characterized by salivation and often chewing movements. These convulsions increase together with paddling movements of the legs, urination and defecation resulting in coma (Roelke-Parker, Munson, Packer, Kock, Cleaveland, Carpenter, O'Brien, Pospischil, Hofmann-Lehmann, Lutz, Mwamengele, Mgasa, Machange, Summers & Appel, 1996, Alexander & Appel,1994). Shedding of the virus begins approximately 7 days post-infection (Appel & Summers, 1995).

1.3 Mortality

Canine distemper is an acute and highly contagious viral disease for many carnivore species. Immunologically naïve populations may experience high death rates. The mortality rates due to CDV infection vary among susceptible species (Appel & Summers, 1995) and could be as high as 100% in ferrets (Von Messeling, Springfield, Devaux & Catteneo, 2003; Williams, Thorne, Appel & Belitsky, 1988).

In domestic dogs mortality rates will largely depend on the immune status of the animal, ranging up to 50% (Ek-kommonen, Sihvonen, Nuotio, Pekkanen, Rikula, 1997). Outbreaks in African wild dogs have led to mortality rates up to 95% (Van der Bildt, Kuiken, Visee, Lema, Fitzjohn & Osterhaus, 2002). However, Serengeti's lion population experienced a decline of 30% in 1994 (Harder, Kenter, Appel, Roelke-Parker, Barret, Osterhaus, 1996).

1.4 Epidemiology

Canine distemper virus infection has been reported in eight of the eleven families of the Order *Carnivora* including members of the families *Ailuridae, Canidae, Hyaenidae, Mustelidae,* (Frölich, Czupalla, Haas, Hentschke, Dedek & Fickel, 2000) *Procyonidae, Ursidae, Viverridae* and *Felidae* (Budd, 1981, Carter, Flores & Wise, 2006), but the virus has also mutated to form phocine distemper, which affects aquatic animals (Kennedy, Kuiken, Jepson, Deaville, Forsyth, Barret, van de Bildt, Osterhaus, Evbatov, Duck, Kydyrmanov, Mitrofanov, 2000).

The transmission of CDV takes place by means of direct contact with aerosol or body fluids from domestic dogs or wild carnivores during the acute phase of the disease.

It can rarely be spread indirectly via food and water contaminated with these fluids (Carter *et al.,* 2006; Hirsch & Zee, 1999) or by humans and their equipment (Van der Bildt *et al.,* 2002).

Canine distemper virus has a high incidence in the world's dog population and spillover that may result from interactions between carnivores may represent an important conservation threat to some species. Different studies have documented cases of canine distemper in different susceptible species all over the world.

1.4.1 Canine distemper in North America

Canine distemper has a broad host range among North American carnivores including the gray fox, raccoons, coyotes, skunks, black bears, black footed-ferrets, badgers and weasels. Canine distemper occurs most often in raccoons throughout the Southeastern U.S. The disease tends to show up every five to seven years, being cyclic in nature. Cases of CD are mostly seen in late fall and early winter, probably because wild populations become stressed by limited availability of food and weather conditions, but can occur all year round.

CDV antibody was present in serum from wild coyotes (*Canis latrans*) in South-eastern Colorado (USA) which was collected and analyzed between 1985 to 1988. The results showed no difference among years or sexes, but the prevalence of CDV antibody was higher (62%) in adults than in juveniles (Gese, Schultz, Rongstad, Andersen, 1991). Another survey conducted in Yellowstone National Park from 1989 to 1993 showed that the prevalence of antibodies in coyotes declined over time from 100% in 1989 to 33% in 1992. The presence of antibodies against CDV was associated with the age of the coyotes (Gese,Schultz, Johnson, Williams, Crabtree, Ruff, 1997).

Other species affected by CDV include the Florida black bear. CDV antibodies have been shown in sera from free-ranging Florida black bears (*Ursus americanus floridanus*) collected between November 1993 and August 1995. The sera were collected from three different geographic areas in Florida (Dunbar,Cunningham, Roof,1998). CD has also been described in free-ranging black footed ferrets in 1985 (*Mustela nigripes*) and in badgers (*Taxidea taxus*) and coyotes (*Canis latrans*) collected in the Meeteetse area (Wyoming) in 1986 (Williams *et al.,* 1988).

1.4.2 Canine distemper in Asia

Canine distemper has been diagnosed in Asia in a free-living masked palm civet (*Paguma larvata*) (Machida, Izumisawa, Nakamura & Kiryu, 1992), but the giant panda (*Ailuropoda melanoleuca)* and the red panda (*Ailurus fulgens) (*McCarthy,Shaw & Goodman, 2007) are also known to be susceptible to CDV (Bronson, Deem, Sanchez & Murray, 2007).

Not only in the United States have bears been shown to be susceptible to CDV. A study in 1996 showed serological evidence of a *morbillivirus* infection in polar bears (*Ursus maritimus*) from Alaska and Russia. There was a significantly greater incidence in the bear samples in Russia. The high prevalence of seropositive bears suggested that the bear *morbillivirus* is endemic in the Arctic regions that were investigated (Follman, Garner, Evermann, & McKeiran,1996).

The first known case of canine distemper in a wild Siberian tiger in the Russian Far East has been confirmed by veterinarians from the New York based Wildlife Conservation Society in 2004 (Bio-Medicine, 2004).

Aquatic animals have also been shown to be susceptible to CDV. A mass die-off of Caspian seals (*Phoca caspica*) occurred in the Caspian Sea from April to August 2000 (Kennedy *et al.,* 2000).

1.4.3 Canine distemper in Europe

Canine distemper outbreaks continue to occur in Europe and caused an epidemic in vaccinated domestic dogs in Finland in 1994 (Ek-Kommonen *et al.,* 1997). In another study in Europe in 1997, between 9 to 13% of the Luxembourg red fox population was estimated positive for antibodies against CDV (Damien, Byron, Martina, Mossong, Osterhaus & Muller, 2002). Also aquatic animals in Europe have experienced outbreaks of the mutated form of CD, phocine distemper virus.

A mass die-off of seals in 1988 was caused by phocine distemper in the Waddensea, the Kattegat, Baltic, North Sea and Irish Sea (Lerwill, Jones, Penrose, 2003).

1.4.4 Canine distemper virus in Africa

Many outbreaks of CD have occurred in Africa in different species of wildlife.

A study among domestic dogs adjacent to the Maasai Mara National Reserve in Kenya in 1989 to 1991 showed an increase in the presence of CD in domestic dogs in 1991. This CDV epizootic in domestic dogs was concurrent with the disappearance of African wild dog packs in the region (Alexander & Appel, 1994; Roelke-Parker *et al.,* 1996).

A captive breeding group of African wild dogs (*Lycaon pictus*) in Tanzania became infected with CDV in December 2000, killing 49 of 52 animals within 2 months (Van der Bildt *et al.,* 2002). The outbreak of canine distemper in the Serengeti severely affected the lion population in 1994. A total of 85% of that lion population had anti-CDV antibodies and the epidemic spread to the Maasai Mara National reserve in Kenya, were several hyenas, bat-eared foxes and leopards were killed (Roelke-Parker *et al.*,1996).

2. Tests for the detection of antibodies reactive to canine distemper virus

There are several serological tests that can be used to detect and determine specific titers against CDV, such as indirect fluorescent antibody test (IFA), ELISA and serum-virus neutralization (SN) tests.

2.1 Indirect fluorescent antibody test (IFA)

The IFA test is a sensitive and versatile test that can detect both IgM and IgG antibody against CDV. It can therefore be used to confirm a diagnosis of acute distemper by detecting IgM antibody, or retrospectively diagnose distemper by detecting seroconversion in paired serum specimens collected during the acute and convalescent phase of the disease.

2.2 Enzyme-linked immunosorbent assay (ELISA)

The ELISA test is a sensitive test that can also detect IgM and IgG antibodies against canine distemper virus in sera. Detection of IgM is useful to diagnose current or recent CDV infections.

2.3 Serum-virus neutralization test (SN)

The serum-virus neutralization (SN) test is the gold standard for detecting antibodies in serum. Antibody titers for CDV can be detected 5-6 days after exposure. The SN test is a highly specific and sensitive test. Generally, end-points are easy to read. Another reason for using the SN test, when dealing with sera from different species, is that there are not always suitable conjugated anti-species antibodies for the species under consideration for use with the IFA test or the ELISA, e.g. hyenas.

3. Results of serological surveys for anti-CDV antibodies in Africa

Several studies have been done during the past few decades examining the prevalence of CDV antibodies in wild carnivores in Africa. Outbreaks of CD in wild carnivores have caused major loses. The results of some of the serological surveys for anti-CDV antibodies in Africa are summarized below.

A study in the KNP in South Africa showed an anti-CDV antibody prevalence of 6% in a study population of lions in 1997. A total of 32 animals had been tested of which two were positive. In the same study, lion sera were tested from Botswana and the Hluhluwe-Imfolozi game reserve in South Africa. The lions in Botswana yielded 14 (26%) positive animals from the 53 that had been tested. However, the lions in Hluhluwe-Imfolozi game reserve yielded no positive animals of the 42 lions, that were tested (Van Vuuren,Styliandes & Durand, 1997).

In South Africa, fatal cases of canine distemper in leopard (*Panthera pardus pardus*) populations have not been reported, although a survey in 1997 showed two of seven leopards positive for distemper virus antibodies in South Africa, and in Botswana one of two leopards was positive for CDV-antibodies (Van Vuuren, *et al.,* 1997).

In the Maasai Mara National Reserve (Kenya), a study done in 2004 revealed a prevalence of 47% in Hyenas (*Crocuta crocuta*) (Harrison, Mazet,Holekamp, Dubovi, Engh, Nelson, Van Horn & Munson, 2004). Near the Maasai Mara National Reserve a survey done among domestic dogs revealed a prevalence of 76% in the same area the wild dog packs disappeared (Alexander & Appel, 1994).

An outbreak of CD in Serengeti National Park during 1994-1995 resulted in major losses lion populations which is an important predator in the ecosystem (Roelke-Parker *et al*.,1996). Canine distemper is therefore an important cause of morbidity and mortality in Africa.

4. The emergence of Transfrontier Conservation areas

The world's first transboundary park was established in 1932, when Wateron National Park (United States) and Glacier National Park (Canada) were merged (UNESCO, 2009). More transfrontier conservation areas (TFCAs) followed worldwide. Currently fourteen (Figure 1) TFCAs exist within the Southern African Development Community (SADC) region, each of them having political support and international agreements. Not only will the TFCAs bring together some of the best and most established wildlife areas in southern Africa, but they also have a cultural importance as it will provide opportunities for tourism. The TFCAs will be of great importance for sustainable economic development in southern Africa, but also promote the conservation of wildlife. Transfrontier Conservation Areas will play a key role in southern Africa for ecotourism development (SANPARKS, 2009; Bengis, 2003; Southern African Peace Parks, 2009). Each TFCA has its own unique (endemic) flora and fauna.

4.1 Kgalagadi TFCA

Southern Africa's first TFCA, the Kgalagadi Park was established and opened in 2000. It is a collaboration between the countries of Botswana, Namibia and South Africa, being a combination of the Kalahari Gemsbok National Park in South Africa and the Gemsbok National Park in Botswana. The Kgalagadi TFCA comprises 3.6 million hectares and consists of red dunes, sparse vegetation and dry riverbeds of

the Auob and Nossob Rivers (SANPARKS, 2009; Southern African Peace Parks Foundation, 2009).

4.2 Richtersveld/Ai-Ais TFCA

The treaty for the IAi-IAis/Richtersveld Transfrontier Park was signed by Namibia and South Africa in 2003 (SANPARKS, 2009; Southern African Peace Parks, 2009). The Richtersveld / Ai-Ais TFCA is situated in an arid mountain desert with rugged kloofs and high mountains. It crosses two countries on both sides of the Orange river and includes the Fish River Canyon.

4.3 Greater Mapungubwe TFCA

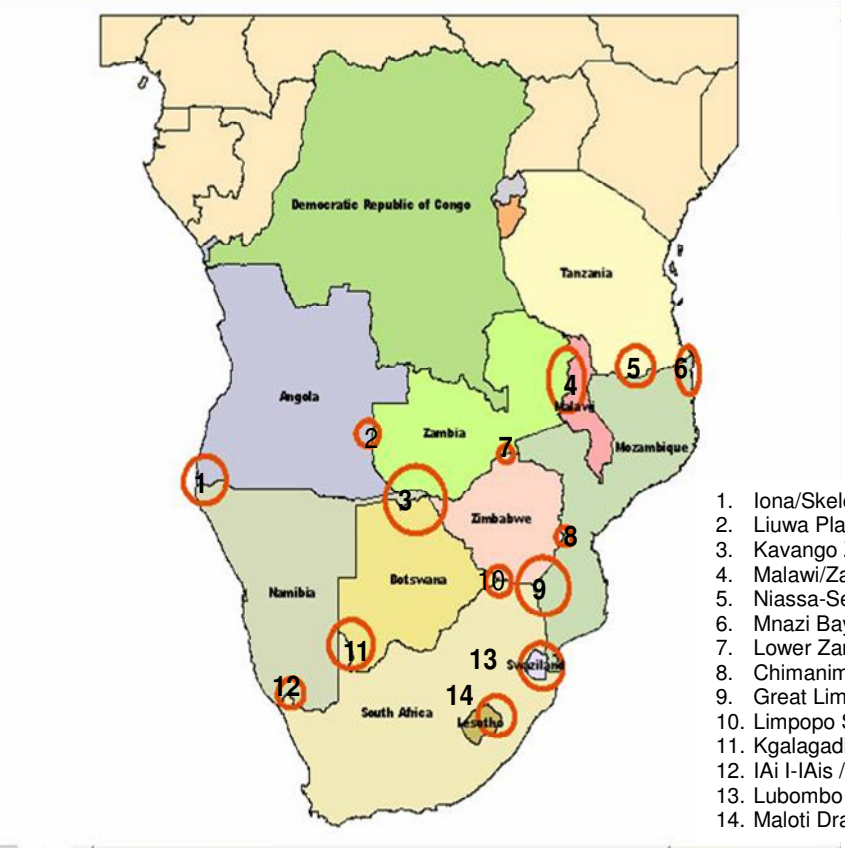
In 2006 an agreement was signed between Zimbabwe, Botswana and South Africa to develop the Mapungubwe world heritage site (SANPARKS, 2009).

4.4 Kavango Zambezi

The Kavango-Zambezi TFCA was established with the signing of a memorandum of understanding in 2006 between Botswana, Zambia, Zimbabwe, Angola and Namibia. It includes the Okavango Delta, Chobe National Park and Zambezi river (Southern African Peace Parks Foundation, 2009).

4.5 Lubombo TFCA

In March 2000 the governments of Mozambique, South Africa and Swaziland signed five protocols for the establishment of the Lubombo Transfrontier Conservation and Resource Area. The Lubombo Conservancy includes Mlawula Nature Reserve, Shewula Nature Reserve, Mbuluzi Game reserve, Hlane National Park and Inyoni Yami Swaziland Irrigation Scheme (IYSIS) (Southern African Peace Parks, 2009). The coastal area included in this TFCA consists of wet lands and low-lying coastal plains.



1. Iona/Skeleton Coast TFCA
2. Liuwa Plain emussuma TFCA
3. Kavango Zambezi TFCA
4. Malawi/Zambia TFCA
5. Niassa-Selous TFCA
6. Mnazi Bay-Quirimbas TFCA
7. Lower Zambezi-Mana Pools TFCA
8. Chimanimani TFCA
9. Great Limpopo TFCA
10. Limpopo Shashe TFCA
11. Kgalagadi Transfrontier Park
12. IAi I-IAis /Richtersveld TFCA
13. Lubombo TFCA
14. Maloti Drakensberg TFCA

Figure 1 Transfrontier Conservation Areas in southern Africa

4.6 Maloti/Drakensberg TFCA

In 2001, a memorandum was signed by Lesotho and South Africa for the establishment of the Maloti-Drakensberg Transfrontier Conservation and Development Area (Southern African Peace Parks, 2009). Including the uKhahlamba Drakensberg World Heritage Site, the Maloti/Drakensberg TFCA is based in a mountainous area and is an important centre for communities of plants and animals that favour montane ecosystems.

4.7 Great Limpopo TFCA

The memorandum of Understanding (MOU) was signed on the 10th of November 2000 by South Africa, Zimbabwe and Mozambique. The GLTFCA could be categorized into four landscape types: lowland plains, granitic plateau, Lebombo mountain range and riverine courses (SANPARKS, 2009; Southern African Peace Parks, 2009).

4.8 Malawi/Zambia TFCA

In 2004 Malawi and Zambia signed an agreement for the Malawi/Zambia TFCA. It includes the Niyka TFCA centered on a high montane grassland plateau and Kasungu/Lukusuzi which is an important area for biodiversity conservation in the Miombo Ecoregion (Southern African Peace Parks, 2009).

4.9 The Chimanimani TFCA

The Chimanimani Transfrontier Conservation Area in the Chimanimani mountains was established by an agreement between Mozambique and Zimbabwe in 2001 (SANPARKS, 2009; Southern African Peace Parks, 2009). It is situated in the Sussundenga district, Manica Province. The landscape can be divided into two types; high mountain and prairies.

4.10 The Iona/Skeleton Coast TFCA

A memorandum of understanding was signed in 2003 by Angola and Namibia to establish the Iona/Skeleton East Coast TFCA. The Iona/Skeleton Coast is based at the coast and consists of desert, sand beaches and rock formations (Southern African Peace Parks Foundation, 2009).

4.11 Liuwa Plain emussuma

The Liuwa Plain emussuma is the result of an agreement between Zambia and Angola. It protects the third largest migratory population of blue wildebeest (*Connochaetes taurinus*) in Africa (Southern African Peace Parks Foundation, 2009).

4.12 Lower Zambezi-Mana Pools

This Transfrontier park is situated in Zambia and Zimbabwe and lies in the Zambezi valley (Southern African Peace Parks Foundation, 2009). It includes one of world's biggest hippo and crocodile populations.

4.13 Niassa - Selous

The Niassa - Selous transfrontier park is a collaboration between Mozambique and Tanzania (Southern African Peace Parks Foundation, 2009). The ecosystem exists of savannahs, forests, wooded grasslands, wetlands and covers approximately 150 000 km^2.

4.14 Mnazi Bay-Quirimbas Transfrontier Marine Conservation Area

The Mnazi Bay-Quirimbas Transfrontier Marine Conservation Area is a collaboration between Mozambique and Tanzania. It has a high coral diversity and the Ruvuma dunes system (Southern African Peace Parks Foundation, 2009).

5. Opportunities and obstacles faced by TFCAs

The ambitious challenge of Transfrontier Conservation Areas is to combine conservation, environmental protection, tourism and economic development. The advantages that follow can be described as;

1. Enhancement of transnational collaboration and co-operation among the countries through the establishment and management of the TFCA.

2. Stimulation of eco-tourism by creating recreation opportunities, which will encourage regional socio-economic development, for example improvement of educational facilities, infrastructure and creation of jobs.

3. Enhancement of conservation and natural ecological processes and therefore stimulation of the natural movements of the animals. It may for example open up the migratory routes of the herds of African elephants and it may reverse the loss of bio-diversity (Mbeki, 2002; Cason, 2003).

Since the late 1950s, disease control has often included fences to keep wildlife (particularly African buffaloes) separated from livestock. Corridors can serve as biological bridges for wildlife, vectors and their pathogens such as foot-and-mouth disease (FMD) (Osofski, Cleaveland, Karesh, Kock, Nyhus, Starr & Yang, 2005; Bengis, 2003). Therefore a thorough risk assessment of diseases should be made before areas are connected.

One of the key-issues that need to be dealt with is the presence of approximately 20 000 people, living inside the Limpopo national park, together with their livestock and domestic dogs and cats (Bice, 2004). Infectious diseases transmitted from these domestic animals might cause a threat to wildlife. Especially infected domestic dogs living on the boundaries of the conservation areas could represent a risk for transmitting CDV, rabies and other diseases when having contact with wildlife.

The African dog population specifically represents a high risk for spreading CDV, because of high numbers of unvaccinated, reproductively-active dogs that expose the wildlife resources to CDV (Leisewitz,Carter, Van Vuuren, Van Blerk, 2001). Due to poverty, high numbers of these domestic dogs in Africa are not vaccinated.

Domestic dogs in some parts of Tanzania were considered to be the source of infection with canine distemper virus in the Serengeti lions in 1994 (Cleaveland, Appel, Chalmers, Chillingworth, Kaare & Dye, 2000). The outbreak in Serengeti killed over a 1000 out of 3000 lions (Harder, Kenter, Appel, Roelke-Parker, Barret & Osterhaus, 1995; Morrel, 1995).

Disease outbreaks have always been an issue worldwide, not only for the immense impact it can have on the specific animal populations, but also on the conservation status of the animal. In Africa the main diseases of concern include bovine tuberculosis, bovine brucellosis, foot-and-mouth disease, theileriosis, canine distemper, rabies, malaria, anthrax, trypanosomosis and African swine fever. Most of these diseases can be transmitted by wildlife or domestic animals. These diseases, except for malaria, are either carried by wildlife or can cause clinical disease in wildlife.

Bovine Tuberculosis (BTB) is a contagious zoonotic disease which is present in the KNP in different species (R. Bengis, state veterinarian, personal communication) When merging the conservation areas, the possibility exists that that TB might be spread to other areas when infected animals are migrating.

5.1 Expected activities of the TFCAs

Future activities of the TFCAs include ongoing relocation of wildlife species. Approximately 6 000 animals of different wildlife species have already been relocated over a period of three years, in the Greater Limpopo transfrontier park, including 1000 elephants. Other activities of TFCAs include community development programmes, the establishment of wildlife sanctuaries, the deployment of qualified field rangers, de-mining of certain areas that were previously involved in the Mozambican civil war according to international de-mining standards, and the drafting of tourism and management plans (SANPARKS, 2009; Southern African Peace Parks, 2009).

6. Marakele National Park

The Marakele National Park, situated in the Limpopo province, was proclaimed in 1994 and known as the Kransberg National Park. It is situated in the middle of the Waterberg mountains and it forms a transitional zone between the dry western and moist eastern regions of South Africa. Marakele's animal population includes elephant, black and white rhino and leopard, but also large predators such as brown hyena, leopard and lion. Marakele also borders with other (private) reserves.

In 2003 an agreement was reached between Marakele National Park and Welgevonden Game Reserve for removal of the fence between them. However, this has been delayed due to the pending outcome of research on theileriosis in African buffaloes (*Syncerus caffer*) in Marakele National Park (Marakele National Park, 2007).

MATERIALS AND METHODS

1. Sera

Large carnivore sera that were tested during this survey were collected opportunistically in the Kruger National Park (KNP) and Marakele National Park (MNP) and included lions, African wild dogs, hyenas and a leopard.The Marakele Park and Kruger National Park are situated in two different provinces in South Africa. The sera were collected opportunistically during 1998, 2000, 2004, 2007 and 2008. Samples were collected randomly and not on the basis of age, location or sex.

The sera were sent by courier from the Faculty of Veterinary Science, University of Pretoria (South Africa) to the University of Utrecht (The Netherlands). Sera were stored frozen in the serum bank of the Kruger National Park. After arriving at the University of Utrecht the samples were stored in a cooler at 4 ℃ for two weeks before testing. The samples were numbered and listed (see appendix).

Sera were inactivated for 30 minutes at 56 ℃ prior to testing. The sera were divided in 4 groups namely, lion (n=198), hyena (n=33), wild dog (n=23) and leopard (1 sample).

2. Virus

The virus used in the SN test was the Onderstepoort strain (passage X+1 12-11-2008 NS-272-97). The virus was titrated to determine the 50% tissue culture infectious dose ($TCID_{50}$) by means of the Reed and Muench method (Reed and Muench, 1938) to enable the use of a viral suspension in the test that contains approximately 100 to 300 infective doses. The $TCID_{50}$ of the virus stock was $10^{4,5}$ $TCID_{50}$/ml. Virus controls were included in every test.

3. Cell culture and media

Vero cells were used in the serum neutralization test as indicator cells. Vero cells are kidney cells derived from the African Green monkey (*Cercopithecus aethiops*). Cells were grown in 75 mm^2 flasks in Dulbecco's modified Eagles medium (DMEM) with 5% foetal calf serum (FCS) and 500 µl / 500ml penicillin/streptomycin.

4. Serum virus neutralization test

The procedure was performed using a standard protocol as previously published with minor modifications (Appel & Robson, 1973). A 1:5 dilution of the test serum was made in PBS+(Mg/Ca) and inactivated for 30 minutes in a waterbath at 56˚C. Two-fold dilutions of sera in DMEM, containing 5% foetal calf serum, were made starting from 1:5 to 1:1280. Serum dilutions of 1:5, 1:10, 1:20, 1:40 were used in this project.

The following concentrations were used for the back titration: 100, 10, 1, 0.1, 0.01, & 0.001 $TCID_{50}$.

Positive and negative control dog sera were included in each micro-titer plate and were provided by H. Egberink, University of Utrecht, the Netherlands. The positive sample was obtained from a dog that suffered from canine distemper. The negative sample was from a specific pathogen-free dog (SPF-dog).

To obtain a 100 $TCID_{50}$ viral suspension, the stock virus was diluted in DMEM containing 5% foetal calf serum. For the virus control a series of four, ten-fold dilutions were made from the 100 $TCID_{50}$ antigen. The virus suspension was added 1:1 to the serum dilution. The solution was mixed and incubated for an hour at 37 ℃ and 5% CO_2. One hundred µl of the 100$TCID_{50}$ antigen was added to all wells containing the diluted test sera.

The virus control was set up as follows: One hundred µl DMEM containing 5% foetal calf serum was added to all the wells. Then 100 µl of the 100 $TCID_{50}$ virus was added to the first two columns and 100 µl of the four dilutions was added accordingly to the remaining four columns, starting with the highest virus dilutions.

The plates were incubated for one hour at 37 ˚C in a humid atmosphere of 5% CO_2 in air. The Vero cells were trypsinized and 80 µl in the correct concentration was added to all the wells.

The cell control was set up in duplicate rows as follows: 200 µl MEM containing 5% foetal calf serum and 80 µl of the cell suspension. The plates were incubated at 37 ˚C in a humid atmosphere of 5% CO_2 in air and observed and recorded daily.

5. Recording of results

Sera were tested in triplicate, and the multi-well plates were observed daily for 5-6 days. A sample was deemed positive when there was a reduction of more than 50% of the cytopathic effect in at least two of the 3 wells.

Data analysis

The prevalence of CDV seropositivity, with 95% binomial exact confidence intervals, was calculated for each species, and within species by age group (juvenile/sub-adult/adult) and sex (male/female). For lions, seroprevalence was also calculated for each region of the Kruger National Park and adjacent reserves (South/Central/North/Far North). Seroprevalence was compared between species, and within species between age groups, sexes and regions using Fisher's exact test. For the lion samples, in order to adjust for confounding, the three predictors "age group", "sex" and "region" were then included in a multiple logistic regression model with CDV seropositivity as the outcome. The fit of the model was assessed using the Hosmer-Lemeshow goodness-of-fit test. The significance level used for all analyses was $\alpha = 0.05$. All statistical tests were performed using Stata 10.1 (StataCorp, College Station, TX, U.S.A.).

RESULTS

Prevalence rates by species

A total of 198 lion sera (see appendices) were tested of which 69 had neutralizing antibodies, (Figure 2) yielding a prevalence of 34,8% seropositivity with a 95% exact binomial confidence interval of 28.2% to 41.9% (Table 1).

Table 1 Prevalence rate in lions in KNP

Species	Variable	Level	*N*	% positive	95% binomial exact C.I.
Lion		Overall	198	34.8	28.2, 41.9
	Age	Cub	1	0	.0, 95
		Juvenile	21	61.9	38.4, 81.9
		Sub-adult	26	38.5	20.2, 59.4
		Adult	119	32.8	24.4, 42.0
	Sex	Female	99	34.3	25.0, 44.6
		Male	74	40.5	29.2, 52.6
	Location	Southern KNP	68	33.8	22.7, 46.3
		Central KNP	47	42.5	28.2, 57.8
		Northern KNP	23	30.4	13.2, 52.9
		Far north KNP	16	31.2	11.0, 58.7

Sera from 33 hyenas were tested and yielded 25 samples with neutralizing antibodies (Figure 2). The prevalence was 75.8% with a 95% exact binomial confidence interval of 57.8% to 88.9% (Table 2).

Table 2 Prevalence rates in hyena in KNP

Species	Variable	Level	*N*	% positive	95% binomial exact C.I.
Hyena		Overall	33	75.8	57.7, 88.9
	Age	Juvenile	1	0	97.5
		Sub-adult	10	70	34.8, 93.3
		Adult	22	81.8	59.7, 94.8
	Sex	Female	19	89.4	66.8, 98.7
		Male	14	57.1	28.9, 82.3

The 23 sera collected from wild dog yielded 18 samples with neutralizing antibodies (Figure 2) and the one leopard sample was similarly positive. The prevalence of CDV antibodies in wild dogs was 78.3% with a 95% exact binomial confidence interval of 56.3% to 92.5% (Table 3).

Table 3 Prevalence rates in wild dogs in KNP

Species	Variable	Level	*N*	% positive	95% binomial exact C.I.
Wild dog		Overall	23	78.2	56.2, 92.5
	Age	Juvenile	8	11.7	47.3, 99.7
		Adult	15	11.4	44.9, 92.2
	Sex	Female	8	62.5	24.4, 91.4
		Male	14	85.7	57.1, 98.2

To compare these results statistically, a cross-tabulation and the Fisher's exact test was used between lion and hyena. Lion had 65.15% negative and 34.85% positive samples for neutralizing antibodies. Hyena had 25% negative and 75% positive samples. In total (hyenas, lions and wild dogs) 58.97% animals were negative and 41.03% positive.

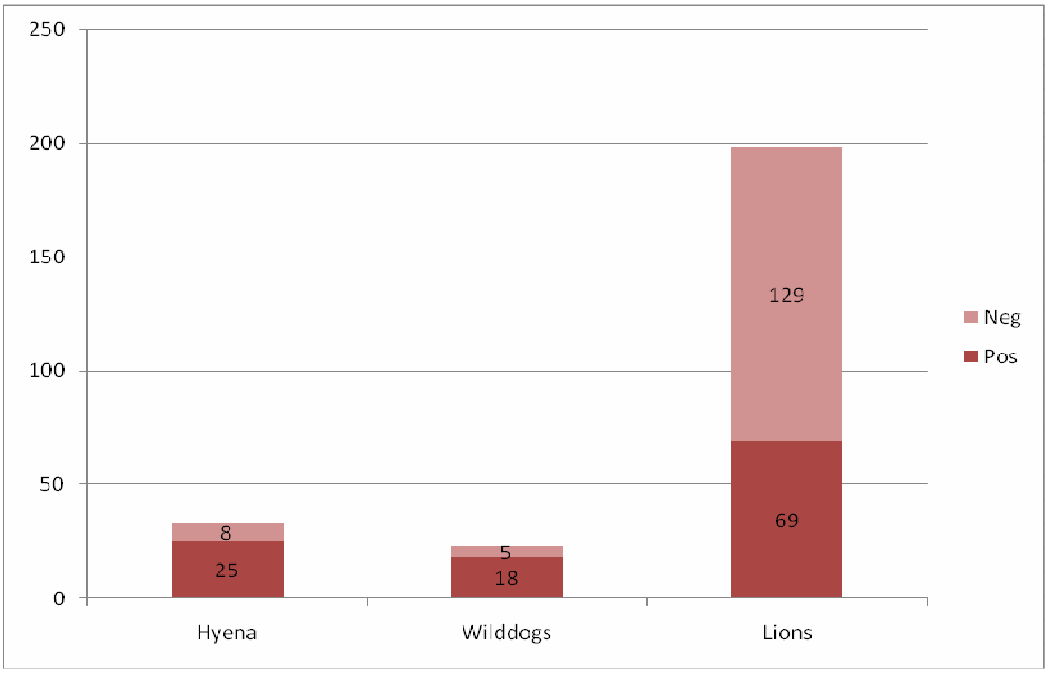


Figure 2 Prevalence in lions, hyenas and wild dogs in KNP and Marakele Park

The difference in prevalence between lions and hyenas was highly significant ($P < 0.001$).

To compare the results of the lion and wild dog statistically, a cross-tabulation and the Fisher's exact test was used between lion and wild dog. Lion yielded 65.15% negative and 34.85% positive samples. Hyena yielded 21.74% negative and 78.26% positive samples. In total 60.63% of the animals had serum samples without neutralizing antibodies and 39.37% had sera with neutralizing antibodies.

There was no significant difference between hyena and wild dog ($P > 0.999$).

Prevalence rates by locations

The southern region of the KNP had 68 positive lions with neutralizing antibodies for CDV. The prevalence of CDV antibodies in lions was 33.8% with a 95% exact binomial confidence interval of 22.8% to 46.3%.

The central region of the KNP had 47 lion for with neutralizing antibodies. The prevalence of CDV antibodies in lion was 42.6% with a 95% exact binomial confidence interval of 28.2% to 57.8%.

The northern region of the KNP had 23 positive cases. The prevalence of CDV antibodies in lion was 30.4% with a 95% binomial confidence interval of 13.2% to 52.9%.

The far north region had 16 positive lions for CDV antibodies. The prevalence was 31.2% with a 95% confidence interval of 11% to 58.7%. Fisher's exact test showed no significant differences between any of the locations.

Prevalence rates by age

To compare the results of the difference in the age of hyena statistically, a cross-tabulation and Fisher's exact test was used. There was no significant difference between ages ($P = 0.248$).

To compare the difference in the age of lions statistically, a cross-tabulation and Fisher's exact test were used. The significance of comparisons between ages was found as follows: juveniles versus sub-adults: $P = 0.147$, juveniles versus adult:

$P = 0.014$ and sub-adults versus adult: $P = 0.649$.

The wild dogs showed no difference between ages ($P = 0.621$).

Prevalence rates by gender

By comparing the difference of gender of lions statistically in a cross-tabulation and Fisher's exact test it was found that there was no significant differences between sexes ($P = 0.248$).

The wild dogs showed no significant difference between sexes ($P = 0.309$).

To compare the difference in sex of hyenas statistically a cross-tabulation and Fisher's exact test were used. There was a marginally significant difference between sexes: 89.5% for females versus 57.1% for males ($P = 0.047$).

Confounding

A multiple logistic regression model was used for the three predictors "age", "sex" and "location" in order to adjust for potential confounding effects. After adjusting for confounding, it was shown that the odds ("risk") of seropositivity decreases with increasing age (odds ratios for age=3 and age=4 are less than one), and for adults it is significantly less than for juveniles (OR = 0.199, 95% CI: 0.07, 0.60; $P = 0.004$).

The odds of seropositivity are significantly lower in locations South, North and Far North compared to central (Table 4).

Table 4 Effects of age, sex and location on the risk of seropositivity to canine distemper virus in 138 serum samples collected from lions in the Kruger National Park and adjacent reserves, 1998-2008

Variable	Level	Odds ratio	95% C.I. (*OR*)	*P*
Age	Adult	1*	–	–
	Sub-adult	2.78	0.99, 7.82	0.053
	Juvenile	5.01	1.67, 15.1	0.004
Sex	Female	1*	–	–
	Male	1.46	0.70, 3.06	0.313
Location	Central	1*	–	–
	South	0.39	0.17, 0.93	0.034
	North	0.20	0.06, 0.70	0.012
	Far north	0.23	0.05, 0.98	0.046

*Reference level
Hosmer-Lemeshow $\chi^2(7 \text{ d.f.}) = 1.06$ ($P = 0.994$)

DISCUSSION

The aim of this study was to estimate the prevalence of CDV antibodies in carnivores from the Kruger National Park and the Marakele National Park. There was no suspicion of canine distemper in these animals, as clinical CD in free-ranging carnivores has not previously been documented in the KNP nor the MNP. However, 6% of lions were seropositive for CDV in a survey done on a limited number of them in the KNP in 1998 (Van Vuuren, *et al.*, 1997).

The hypothesis still stands that clinical canine distemper is not widespread in large wild carnivores in the KNP and MNP and will not represent a risk to carnivores in the GLTFCA when free movement of animals takes place.

However, there is a significant prevalence of CDV-antibodies in the large carnivores of the KNP indicating mainly inapparent infection. It confirms that the virus is present in the KNP and when merging the KNP with other conservations areas, it could spread.

For this study, the serum-virus neutralization test was selected, as it is the gold standard for detecting canine distemper virus antibodies. The SN test is a highly specific and extremely sensitive test and is considered the most reliable serological procedure, and is less prone to variation and less subjective in its interpretation. Generally, end-points are easy to read. The principle of the test is based on the fact that the demonstrable replication and activity of the virus, namely cytopathic effect in cell culture can be inhibited by specific viral antibody. Another reason for using the SN test was the fact that there is no conjugated anti-species antibody against hyena IgG antibodies available for use in other tests such as ELISA or IFA.

In this study it was noticed that some serum samples from hyenas did not result in complete (100%) protection of the virus-infected cell cultures, but that the anti-CDV antibodies in the serum only produced partial neutralization. This phenomenon has previously been documented. It is known that for some viruses 100% neutralization

of cytopathic effects (CPV) is never achieved, even when using hyper-immune serum (Potgieter & Aldridge, 1977). Therefore, when serum samples resulted in a reduction of more than 50% of the CPE, it was regarded as positive for the presence of neutralizing antibodies.

Even though, some of the sera showed some toxicity for cells in wells at a dilution of 1:5, the antibody titre could still be determined in wells with higher serum dilutions and expressed as positive or negative for the presence of neutralizing antibodies. In a similar study in 2004 with hyena sera, the authors chose a serum dilution of 1:10 as the lowest dilution to express neutralizing antibody titers (Harrison *et al*., 2004). In the current study a serum dilution of 1:5 was selected as the cut-off point for expression of neutralizing antibody titers. The decision was based on the clear neutralizing effects that could still be observed at a serum dilution of 1:5.

The Fishers's exact test did not show significant differences in lions between any of the locations (South, Central, North and Far North of the KNP). Although the central regions seemed to have a higher prevalence, it was not significant.

A notable feature of the results is the fact that positive samples were found in all 4 species tested. The chance is limited, but not ruled out, that any of these species had contact with each other as the samples have been taken randomly from different locations and years. However, the wild dog sera are all, except one, from the same location. Wild dogs are known to live in packs and could therefore have been infected by group-members or they could also have had contact with domestic dogs on the fringe or periphery of Marekele Park.

The lion sera were collected randomly from all over the Kruger National Park. Lions have a very variable social organization and may switch their lifestyle. Lions may form prides or become nomads. Prides consist normally of females (five to six members) or males (two to four). Nomads are formed by males who range widely and move out sporadically. Their lifestyle could contribute to the spread of diseases among group members. The samples were randomly collected and contained different sexes. The lions showed no significant differences between sexes (P= 0,248).

Hyenas showed a marginal difference between sexes; 89.4% for females versus 57.1% for males (*P*=0,047). Two hyenas were tested more than once. The antibody titre of hyena no. 009 declined more than two dilutions over 5 months, which is an expected decline. The other two re-captures (hyena no. 007) were too close to make a conclusion.

The hyena sera were all collected in the Skukuza region (South) in KNP, which makes it possible to be spread in their own clan. It has furthermore been postulated that hyenas in that area are close enough to domestic dogs on the western border of the KNP to make contact with them during nightly migrations (L. Bolton, unpublished results, 2009).

A study in Zimbabwe in 2002 investigated the interaction of domestic dogs as predators and/or preys of wild carnivores such as hyena, leopard and lion. It was shown that spotted hyena, lion and leopard preyed on dogs in the Gokwe communal land. The study concluded that predation provides the ideal circumstances for disease transmission as rabies and probably canine distemper were prevalent in the study area (Butler, Du Toit, Bingham, 2004). Leopards, however, live solitary and it is not known how this carnivore could have become exposed to CDV in the KNP. However, on occasion they also prey on dogs.

Clinical distemper has not previously been documented in the KNP (Van Vuuren, *et al*., 1997). However, due to its large geographical area, it cannot be excluded that the disease occurred in isolated incidents in single animals. A retrospective study in 2008 reconstructed the history of CDV exposure in the Serengeti and Ngorongoro lion populations. It showed that climatic conditions might trigger epidemics with greater mortality by several infectious agents than due to single pathogens as temporal and spatial convergence of several infectious agents under certain environmental conditions could favour their transmission (Munson, Terio, Kock, Mlengeya,Roelke, Dubovi, Summers, Sinclair & Packer, 2008). This might be one of the reasons why clinical distemper in wild carnivores has so far been absent in the KNP.

The odds of seropositivity are significantly lower in locations South, North and Far North compared to central in the KNP. The reason for this difference is that there was confounding between location and age. There were more adults and fewer juveniles in the central region. Juveniles had a higher prevalence of seropositivity, so the fact that there were fewer juveniles in the central region made the prevalence in the central region artificially lower. Thus, an adjustment for age was implemented, and the odds of seropositivity in the central region became significantly higher than all the other regions. In this study species have been compared from the KNP and MNP to show the difference in positive cases, however the parks are far away from eachother.

CONCLUSION AND RECOMMENDATIONS

Transfrontier Conservation Areas represent the future challenge of African countries to combine conservation, environmental protection, tourism and socio-economic development. From an environmental point of view the protection of conservation areas and animal species by opening up fences is enormous.

Creating these conservation areas will create a huge biodiversity of flora and fauna. It will make it possible to provide an enormous area for species that are threatened such as the wild dog.

In this study canine distemper virus has been shown to circulate among the lions, leopards, wild dogs and hyenas in the KNP, and the wild dogs in MNP and could therefore represent a risk when merging new areas to create the GLTFCA, or when translocating animals to other conservation areas.

Monitoring and surveillance are important to prevent outbreaks of disease, and risk assessment is an important tool to determine the risk for spread of a disease when merging conservation areas. The prevalence rates obtained in this study from sera collected between 1998 and 2008 should provide meaningful information for risk assessors and risk managers when considering the risk for the spread of canine distemper virus and deciding whether control measures should be instituted.

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APPENDICES

Sera Carnivores Kruger National Park, South Africa

Nr. lab.	Animal nr.	Specie	Date Blood sample taken	Age	Sexe	Location	SN Titer
1	C5706	Lion	19/06/08	Adult	Male	Bangu	Neg
2		Lion	26/04/07	9 years	Female	Ngirivane	10
3	7A06	Lion	19/06/08	Adult	Male	Bangu	Neg
4	2857	Lion	09/05/08	Not known	Not known	Sabie sand nature reserve	Neg
5	15856(C)	Lion	19/06/08	Adult	Female	Bangu	Neg
6	2A4F	Lion	09/05/08	Not known	Not known	Sabie Sand Nature Reserve	Neg
7	3622B	Lion	19/06/08	Adult	Female	Bangu	Neg
8	1C54	Lion	09/05/08	Not known	Not known	Sabie Sand Nature Reserve	Neg
9	D1F	Lion	05/11/07	4 years	Female	Nwarihlangari	Neg
10	E1463	Lion	19/06/08	Adult	Female	Bangu	Neg
11	4AOA	Lion	09/05/08	Not known	Not known	Sabie sand nature reserve	Neg
12	F2C	Lion	06/11/07	9 years	Female	Nwarihlangari	Neg
13	3C10	Lion	09/05/08	Not known	Not known	Sabie Sand Nature Reserve	Neg
14		Lion	27/02/07	10 years	Female	Mativulungu loop	Neg
15	O.OOE+ OO OE21	Lion	09/05/08	Not known	Not Known	Sabie Sand Nature Reserve	Neg
16	F43	Lion	06/11/07	12 months	Female	Nwarihlangari	Neg
17		Lion	19/06/08	Adult	Female	Bangu	Neg
18	313	Lion	06/11/07	10 years	Female	Nwarihlangari suid	5
19	4a68	Lion	08/05/09	Not known	Not known	Sabie sand nature reserve	Neg
20	-	Lion	07/12/05	9 years	Male	Tsokwane	Neg
21	34978	Lion	19/06/08	Adult	Female	Bangu	Neg
22	1570	Lion	09/05/08	Not known	Not known	Sabie Sand Nature Reserve	Neg
23	086^{E}	Lion	09/05/08	Not known	Not known	Sabie sand nature reserve	Neg
24	02A5F	Lion	19/06/08	Adult	Female	Bangu	Neg
25	13F1E	Lion	19/06/08	Adult	Male	Bangu	Neg
26	66D	Lion	06/11/07	14 months	Male	Nwarihlangari	Neg
27	463B	Lion	09/05/08	Not known	Not known	Sabie Sand Nature Reserve	Neg
28	C57	Lion	06/11/07	14 months	Male	Nwarihlangari	10
29	06/62 lion1	Lion	01/04/06	Sub-adult	Female	Kalagadi NP	Neg
30	06/08	Lion	12/02/06	Adult	Female	Satara	10
31	06/07	Lion	12/02/06	Adult	Male	Satara	10
32	06/80	Lion	05/03/06	6-7 years	Male	Satara	10
33	06/82 lion3	Lion	05/04/06	Adult	Female	Satara	10
34	06/34 lion1	Lion	04/10/06	Adult	Female	Satara	10
35	06/376	Lion	26/09/06	Adult	Male	Satara	Neg
36	00/1974	Lion	17/12/00	Adult	Female	Sabie Park	20

37	00/520 lion3	Lion	27/06/08	Adult	Male		Neg
38	2 06/63	Lion	01/04/06	Sub-adult	Female	Kalagadi NP	>40
39	00/187	Lion	13/04/00	Adult	Male	State Vet Bomas	Neg
40	00/40	Lion	24/01/00	16 months	Male	Renoster koppies	20
41	00/1969	Lion	04/12/00	Adult	Female	Renoster koppies	Neg
42	00/159	Lion	27/06/00	Adult	Male	Lisben Estates	Neg
43	06/83 lion 4	Lion	05/04/06	Adult	Female	Crocodil bridge	5
44	05/637	Lion	17/11/05	Adult	Male	Skukuza	10
45	98/1155	Lion	04/12/98	Nb	Nb	Nb	Neg
46	98/48	Lion	03/04/98	Young/ sub-adult	Female	Nuyeshi	Neg
47	98/262	Lion	02/09/98	Adult	Female	Mthetamusha	BAC
48	05/179	Lion	21/04/05	Sub-adult	Male	Skukuza Bomas	20
49	06/633 lion3	Lion	01/11/05	Adult	Male	Satara	10
50	98/1150	Lion	04/12/98	NB	NB	NB	Neg
51	05/645	Lion	27/12/05	Adult	Male	Skukuza	Neg
52	05/221 lion2	Lion	19/05/05	Adult	Female	Satara	10
53	08/582	Lion	12/09/05	Sub adult	Female	Satara	Neg
54	98/142	Lion	25/06/98	Adult	Female	Sabie Park	Neg
55	98/1151	Lion	98/12/04	Nb	Nb	Nb	BAC
56	98/1149	Lion	98/12/04	Nb	Nb	Nb	Neg
57	98/156	Lion	06/07/98	Adult	Male	Nb	Neg
58	98/200	Lion	30/07/98	Adult	Male	Nb	5
59	98/1154	Lion	04/12/98	Nb	Nb	Nb	10
60	98/1152	Lion	98/12/04	Nb	Nb	Nb	5
61	98/1156	Lion	98/12/04	Nb	Nb	Nb	Neg
62	98/145	Lion	98/06/26	Adult	Male	Bubuwindmill	Neg
63	98/49	Lion	98/04/03	Young	Male	Nb	Neg
64	98/261	Lion	98/09/02	Young/ sub adult	Female	Nb	Neg
65	04/455 lion47	Lion	09/09/04	Not known	-	Lower Sabie	Neg
66	04/418 lion25	Lion	08/09/04	Juvenile	Male	Nangazwane	20
67	04/0412 lion19	Lion	08/09/04	Juvenile	Female	Crocodile bridge Nogomon.	20
68	04/460 lion52	Lion	09/09/04	Not known	Not known	Lower sabie	Neg
69	04/420 lion27	Lion	08/09/04	young adult	Male	Nangazwane	Neg
70	98/138	Lion	18/06/98	Adult	Male	Mhlambamdu	20
71	98/215	Lion	04/08/98	Nb	Nb	Nb	20
72	98/199	Lion	30/07/98	Adult	Male	Nb	Neg
73	98/259	Lion	02/09/98	Adult/cub?	Male	Nb	>40
74	98/1157	Lion	07/12/98	Juvenile	Female	Nb	20
75	98/1153	Lion	04/12/98	Nb	Nb	Nb	Neg
76	98/260 t	Lion	02/09/98	Adult	Female	Nb	Neg
77	98/22	Lion	08/01/98	Adult	Male	Nb	Neg
78	07/809 56	Lion	17/09/07	4 years	Female	Nkongoma road	Neg
79	07/53	Lion	02/04/07	3 yrs	Male	Lower Sabie	5
80	07/788 lion1	Lion	05/09/07	Adult	Female	Satara	Neg
81	07/815 6278	Lion	19/09/07	2 years	Male	Lower Sabie	10
82	07/357	Lion	15/07/07	Adult	Male	Satara Thompson camp	20
83	07/813	Lion	17/09/07	8 years	Male	Ngongoma road	Neg

	2075						
84	07/08/04 oe 07	Lion	17/09/07				5
85	07/08	Lion	24/01/07	Adult	Female	Satara	Neg
86	07/811 83b	Lion	17/09/07	Not known	Not known	Nkongoma road	Neg
87	07/860 218	Lion	17/09/07	18 months	Female	Nkongoma road	Neg
88	07/356	Lion	15/07/07	Adult	Female	Satara Thompson Camp	Neg
89	07/08/06 lion1	Lion	18/09/07	3 years	Male		Neg
90	07/812 c28	Lion	17/09/07	5 years	Female	Nkongoma road	Neg
91	07/787 lion2	Lion	05/09/07	Adult	Female	Satara	Neg
92	98/47	Lion	98/04/03	Adult	Female	Nuyeshi	10
93	07/370	Lion	19/02/07	Adult	Male	Satara, S90	Neg
94	04/421 lion2	Lion	08/09/04	Adult	Male	Nangazwane	Neg
95	04/457 lion49	Lion	09/09/04	5 years	Female	Lower Sabie	Neg
96	07/07	Lion	24/01/07	Adult	Male	Satara	Neg
97	04/449 lion42	Lion	09/09/04	Adult	Male	Lower Sabie	Neg
98	04/425 lion32	Lion	08/09/04	Young-adult	Male	Nangazwane	20
99	04/396 lion7	Lion	08/09/04	Adult	Female	Bob Picket	Neg
100	04/422 lion29	Lion	08/09/04	Young-adult	Female	Nangazwane	5
101	04/454 lion46	Lion	09/09/04	Adult	Female	Lower Sabie	Neg
102	04/446 lion39	Lion	09/09/04	Adult	Female	Lower Sabie	Neg
103	04/445 lion38	Lion	09/09/04	Adult	Female	Lower Sabie	Neg
104	04/450 lion43	Lion	09/09/04	Adult	Male	Lower Sabie	Neg
105	04/444 lion37	Lion	09/09/04	Adult	Male	Lower Sabie	Neg
106	04/448 lion41	Lion	09/09/04	Young adult	Male	Lower Sabie	Neg
107	04/413 lion20	Lion	08/09/04	Juvenile	Female	Crocodile bridge Ngomondwe	10
108	04/459 lion51	Lion	09/09/04	Not known	Not known	Lower Sabie	Neg
109	04/398 lion9	Lion	07/09/04	Young adult	Female	Dzaweni	10
110	04/410 lion17	Lion	08/09/04	Adult	Female	Crocodile bridge Ngomondwe	10
111	04/309 lion3	Lion	27/07/04	Adult	Male	Skukuza	10
112	04/443 lion36	Lion	09/09/04	Adult	Male	Lower Sabie	Neg
113	04/419 lion26 (09)	Lion	08/09/04	Young adult	Male	Nangazwane	Neg
114	04/308 lion2	Lion	27/07/04	Young adult	Female	Skukuza	Neg
115	00/186	Lion	15/04/00	Adult	Male	Thakwane leeupark	Neg
116	00/39	Lion	24/01/00	Adult	Female	Berg en Dal	Neg
117	04/392 lion3	Lion	07/09/04	Adult	Female	Gezambtombi	Neg

118	04/402 lion13	Lion	08/09/04	Juvenile	Female	Dzaweni	Neg
119	04/397 lion8	Lion	07/09/04	Adult	Female	Dzaweni	Neg
120	04/390 lion1	Lion	07/09/04	Young adult	Female	Gezambtombi	Neg
121	04/427 lion34	Lion	08/09/04	Juvenile	Female	Nangzwane	20
122	04/416 lion23	Lion	08/09/04	Adult	Female	Croc. Bridge Ngomondwe	Neg
123	04/394 lion5	Lion	07/09/04	Adult	Male	Gezambtombi	>40
124	04/452 lion45	Lion	09/09/04	Adult	Male	Lower Sabie	10
125	04 417 lion24	Lion	08/09/04	Juvenile	Male	Nangazwane	Neg
126	04 /428 lion35	Lion	08/09/04	Juvenile	Male	Nangazwane	>40
127	04/400 lion11	Lion	08/09/04	Juvenile	Male	Dzaweni	20
128	05/617 lion2	Lion	29/09/05	8 years	Male	Satara	20
129	04/295	Lion	21/07/04	Adult	Male	GG Bomas	>40
130	98/146	Lion	26/06/98	Adult	Female	Bubuwindmill	Neg
131	98/141	Lion	24/06/98	Sub-adult	Female	-	Neg
132	04/601	Lion	19/10/04	2.5-3.5 years	Male	Tswalu	20
133	04/567 lion10	Lion	12/10/04	5-6 years	Female	Bogela North	Neg
134	04/567 lion1	Lion	11/10/04	Adult	Female	Shingwedzi S. Piper.	10
135	04/574 lion8	Lion	11/10/04	Not known	Not known	Mpenza	10
136	04/600	Lion	19/10/04	8 years	Male	Tswalu	5
137	04/603 lion1	Lion	28/10/04	Adult	Male	Nwanetsi E. windmill	5
138	04/575 lion9	Lion	11/10/04	5 years	Female	Mpenza	Neg
139	04/607 lion5	Lion	28/10/04	3 years	Female	Nwanetsi E. windmill	40
140	04/587 lion21	Lion	13/10/04		Female	Nwahitaimbe	10
141	04/604 lion2	Lion	28/10/04	Adult	Male	Nwanetsi E. windmill	Neg
142	04/595	Lion	14/10/04	3 years	Male	Tswalu	40
143	04/596	Lion	19/10/04	4-5 years	Male	Tswalu	>40
144	04/426 lion33	Lion	08/09/04	Juvenile	Female	Nangazwane	20
145	04/456 lion48	Lion	09/09/04	Adult	Female	Lower Sabie	Neg
146	04/423 lion30	Lion	08/09/04	Juvenile	Female	Nangazwane	20
147	04/635	Lion	26/11/04	Adult	Female	Croc. Bridge	Neg
148	04/447 lion40	Lion	09/09/04	Young adult	Male	Lower Sabie	Neg
149	04/582 lion16	Lion	13/10/04	8 years	Female	Nwashitaimbe	Neg
150	04/577 lion11	Lion	12/10/04	Adult	Female	Bogela North	Neg
151	04/399 lion10	Lion	07/09/04	Adult	Male	Dzaweni	Neg
152	04/424 lion31	Lion	08/09/04	Juvenile	Female	Nangazwane	Neg
153	04/403 lion14	Lion	08/09/04	Juvenile	Male	Dzaweni	20

154	04/411 lion18	Lion	08/09/04	Adult	Female	Croc.bridge Ngomondwe	>40
155	04/578 lion12	Lion	13/10/04	6 years	Female	Nwashitaimbe	5
156	04/393 lion4	Lion	07/09/04	Adult	Female	Gezambtombi	20
161	04/391 lion2	Lion	07/09/04	Young adult	Female	Gezambtombi	Neg
162	04/209	Lion	26/05/04	Adult	Male	Skukuza	Neg
163	04/415 lion22	Lion	08/09/04	Adult	Male	Croc. Bridge. Ngomondwe	Neg
164	04/401 lion12	Lion	08/09/04	Juvenile	Female	Dzaweni	Neg
165	04/409 lion16	Lion	08/09/04	Adult	Female	Croc.bridge. Ngomondwe	Neg
166	04/408 lion15	Lion	08/09/04	Adult	Female	Croc.bridge	20
167	04/307 lion1	Lion	27/07/04	Young adult	Female	Skukuza	10
168	04/632	Lion	25/11/04	Sub-adult	Male	Marakele (PTY) LTD	20
169	04/605 lion3	Lion	28/10/04	5-6 years	Female	Nwanetsi E. Windmill	Neg
170	04/569 lion3	Lion	11/10/04	Adult	Female	Biesiesvlei. Dam	Neg
171	04/571 lion5	Lion	11/10/04	18 months	Female	Mpenza	Neg
172	04/585 lion19	Lion	13/10/04	4 years	Female	Nwashitaimbe	Neg
173	04/584 lion18	Lion	13/10/04	2 years	Male	Nwashitaimbe	Neg
174	04/589 lion23	Lion	13/10/04	4 years	Female	Nwashitaimbe	Neg
175	04/586 lion28	Lion	13/10/04	6 years	Female	Nwashitaimbe	Neg
176	04/572 lion6	Lion	11/10/04	18 months	Female	Mpenza	Neg
177	04/593	Lion	19/10/04	Not known	Not known	Nwashitaimbe	Neg
178	04/588 lion22	Lion	13/10/04	3 years	Female	Nwashitaimbe	Neg
179	04/581 lion15	Lion	13/10/04	8 years	Male	Nwashitaimbe	10
180	04/580 lion14	Lion	13/10/04	4 years	Female	Nwashitaimbe	10
181	04/590 lion24	Lion	13/10/04	10 years	Female	Nwashitaimbe	Neg
182	04/568 lion2	Lion	11/10/04	Adult	Male	Biesiesvlei. Dam	Neg
183	04/573 lion7	Lion	11/10/04	Not known	Female	Mpenza	Neg
184	04/594	Lion	19/10/04	8 years	Male	Tswalu	Neg
185	04/606 lion4	Lion	28/10/04	3 years	Female	Nwanetsi E. Windmill	5
186	04/008 lion6	Lion	28/10/04	8 years	Female	Nwanetsi E. Windmill	Neg
187	04/570 lion4	Lion	11/10/04	Sub-adult	Female	Biesiesvlei dam	5
188	04/591 lion25	Lion	14/10/04	6.5 years	Male	Nwashitaimbe	20
189	04/579 lion13	Lion	13/10/04	2 years	Male	Nwashitaimbe	Neg
190	04/596	Lion	19/10/04	45year	Male	Tswalu	Neg
191	04/583 lion17	Lion	13/10/04	Not known	Female	Nwashitaimbe	Neg
192	05/618	Lion	29/09/05	8 years	Female	Satara	10

	lion3						
193	98/47	Lion	03/04/98	Adult	Female	Nuyeshi	Neg
194	08/632 lion2	Lion	01/11/05	Adult	Male	Satara	Neg
195	05/632 lion2	Lion	01/11/05	Adult	Male	Satara	>40
196	05/1223 lion4	Lion	19/05/05	Juvenile	Female	Satara	Neg
197	(08/8581) 05/581 lion1	Lion	12/09/05	Adult	Male	Satara	10
198	08/1620	Lion	18/10/05	Adult	Female	Sweni	20
199	05/644 lion2	Lion	15/12/05	6-7 years	Male	Satara	Neg
200	05/621	Lion	18/10/05	Adult	female	Sweni	Neg
201	05/222 lion3	Lion	19/05/05	Juvenile	Male	Satara	10
202	005/18 lion1	Lion	27/06/08	Adult	Male		Neg
203	05/1492	Lion	28/07/05	Adult	male	Satara	20
204	98/137	Lion	98/06/18	Adult	Female	Bhlambamdu	20
205	05/0606 lion1	Lion	29/09/05	3 years	Female	Satara	20
206	05/220 lion1	Lion	19/05/05	Adult	Female		10
207	05/643	Lion	15/12/05	5 years	Female	Satara	Neg
208	98/258	Lion	NB	Cub	Male	-	Neg
209	08/95	Lion	25/05/08	Adult	Female	GG bomas	Neg
210	08/01	Lion	02/01/08	Adult	Male	-	Neg
211	08/92	Lion	21/05/08	Adult	Female	Lower Sabie	Neg
212	07/09	Lion	02/02/07	Sub-adult	Female	Madikwe NP	5
Wild dogs							
Wd1	07/878 WD12	Wild dog	06/11/07	Adult	Male	Marakele	5
Wd2	07/869 WD 3	Wild dog	06/11/07	Adult	Male	Marakele	10
Wd3	07/883 WD17	Wild dog	06/11/07	Adult	Male	Marakele	20
Wd4	07/877 WD 11	Wild dog	06/11/07	Adult	Male	Marakele	Neg
Wd5	07/870 WD4 j	Wild dog	06/11/07	Juvenile	Female	Marakele	20
Wd6	07/862	Wild dog	01/11/07	Adult	Male		5
Wd7	07/880 WD14	Wild dog	06/11/07	Adult	Male	Marakele	10
Wd8	07/879 WD13	Wild dog	06/11/07	Adult	Male	Marakele	Neg
Wd9	07/872 WD6	Wild dog	06/11/07	Juvenile	Male	Marakele	20
Wd10	07/884	Wild dog	06/11/07	Adult	Female	Marakele	5
Wd11	07/875 WD 9	Wild dog	06/11/07	Juvenile	Female	Marakele	20
Wd12	07/873 WD7	Wild dog	06/11/07	Juvenile	Female	Marakele	Neg
Wd13	07/871 WD5	Wild dog	06/11/07	Adult	Female	Marakele	Neg
Wd14	07/882 WD16	Wild dog	06/11/07	Adult	Female	Marakele	10
Wd15	07/874 WD8	Wild dog	06/11/07	Juvenile	Female	Marakele	20
Wd16	07/876 WD10 00	Wild dog	06/11/07	Juvenile	Male	Marakele	5
Wd17	07/868 WD 2	Wild dog	06/11/07	Juvenile	Female/ male	Marakele	20

Wd18	07/881 WD15	Wild dog	06/11/07	Juvenile	Male	Marakele	>40
Wd19	06/458	Wild dog	02/11/06	Adult	Male	Marakele	>40
Wd20	06/460	Wild dog	02/11/06	Adult	Male	Marakele	20
Wd21	06/461	Wild dog	02/11/06	Adult	Female	Marakele	Neg
Wd22	06/456	Wild dog	02/11/06	Adult	Male	Marakele	>40
Wd23	06/459	Wild dog	02/11/06	Adul	Male	Marakele	5
Wd24	06/457	Wild dog	02/04/06	Adult	Male	Marakele	BAC
Wd25	08/44	Wild dog	03/04/08	Adult	Female	Mashatu	Neg
Hyaena's							
H1	00/73	Hyaena	13/02/00	Sub- adult	Male	Skukuza bomas	10
H2	3	Hyaena	04/11/12	Adult	Female	Skukuza	10
H3	005/21	Hyaena	27/06/00	Adult	Male	Skukuza rest camp	10
H4	00/99	Hyaena	02/03/00	Sub-adult	Male	Skukuza staff village	Neg
H5	001/30	Hyaena	06/03/08	Sub-adult	Male	Bomas	20
H6	001/64	Hyaena	23/03/00	Adult	Female	Bomas	20
H7	002/52	Hyaena	03/05/00	Adult	Male	Stat vet bomas 00/253	Neg
H8	07/36	Hyaena	15/03/07	Sub-adult	Male		20
H9	5	Hyaena	04/11/12	Adult	Female	Skukuza	>40
H10	-	Hyaena	04/11/12	Adult	Male	Skukuza	5
H11	-	Hyaena	05/06/03	Adult	Female	Salitjepad	10
H12	001 101	Hyaena	04/03/08	Adult	Female	Skukuza comalep	10
H13	0042 s	Hyaena	29/01/00	Sub-adult	Female	Skukuza staff village	5
H14	00/167	Hyaena	28/03/00	Adult	Female	Rugby field	10
H15	001/63	Hyaena	22/03/00	Adult	Female	Bomas	10
H16	07/33	Hyaena	14/03/07	Adult	Female	Skukuza	Neg
H17	07/31	Hyaena	13/03/07	Puppy Juvenile	Male	Skukuza	Neg
H18	07/40	Hyaena	15/03/07	Adult	Male	Skukuza bomas	Neg
H19	07/37	Hyaena	15/03/07	Subadult	Male	Skukuza	Neg
H20	08/99	Hyaena	21/06/08	Adult	Female	Skukuza phabeni road	10
H21	08/628	Spotted Hyaena	27/08/08	Adult	Female	Phabeni road	20
H22	07/34	Hyaena	14/03/07	Adult	Female	Skukuza subad	20
H23	07/779	Hyaena	13/08/07	Adult	Male	Skukuza doispan	5
H24	07/32	Hyaena	13/03/07	Adult	Male	Skukuza (5min)	Neg
H25	08/627	Hyaena	27/08/08	Adult	Female	Phabeni road	20
H26	07/41	Hyaena	15/03/07	Subadult	Male	Skukuza boma's	20
H27	7/30	Hyaena	12/03/07	Subadult	Male	Skukuza (5min)	10
H28	07/42	Hyaena	16/03/07	Adult	Male	Skukuza boma's	Neg
H29	08/660	Hyaena	10/09/08	Adult	Female	Phabeni road	20
H30	00/100	Hyaena	31/03/08	Subadult	Female	Skukuza phabeni road	20
H31	08/100	Hyaena	21/06/08	Adult	Female	Skukuza phabeni road	10
H32	08/515	Hyaena	28/01/08	Adult	Female	Crocodil bridge	10
H33	07/802	Hyaena	20/08/07	Adult	Female	Skukuza	5
H34	07/47	Hyaena	15/03/07	Subadult	Female	Skukuza boma's	Neg
H35	07/45	Hyaena	19/03/07	Subadult	Male	Skukuza boma's	20
H36	07/35	Hyaena	14/03/07	Adult	Male	Skukuza doispan	20
Leopard							
Le 1	-	Leopard	08/07/07	9 years	Male	Numbi	5
TOTAL	**274**						

Statistics

```
. bysort species: ci result, b

-------------------------------------------------------------------------------

-> species = lion
-- Binomial Exact
    Variable |        Obs        Mean    Std. Err.       [95% Conf.
Interval]
-------------+-------------------------------------------------------------
      result |        198    .3484848    .0338627        .2823159
.4192678

-------------------------------------------------------------------------------


-> species = hyena
-- Binomial Exact
    Variable |        Obs        Mean    Std. Err.       [95% Conf.
Interval]
-------------+-------------------------------------------------------------
      result |         36         .75    .0721688        .5779704
.8787967

-------------------------------------------------------------------------------


-> species = wilddog
-- Binomial Exact --
    Variable |        Obs        Mean    Std. Err.       [95% Conf.
Interval]
-------------+-------------------------------------------------------------
      result |         23    .7826087    .0860061        .5629693
.9253966


. tab species result if species!=3, r ex

           |        result
   species |       neg        pos |     Total
-----------+----------------------+----------
      lion |       129         69 |       198
           |     65.15      34.85 |    100.00
-----------+----------------------+----------
     hyena |         9         27 |        36
           |     25.00      75.00 |    100.00
-----------+----------------------+----------
     Total |       138         96 |       234
           |     58.97      41.03 |    100.00

           Fisher's exact =                 0.000
```

. tab species result if species!=2, r ex

species	result neg	pos	Total
lion	129	69	198
	65.15	34.85	100.00
wilddog	5	18	23
	21.74	78.26	100.00
Total	134	87	221
	60.63	39.37	100.00

Fisher's exact = 0.000

. bysort location: ci result if species==1, b

-> location = south

-- Binomial Exact

Variable	Obs	Mean	Std. Err.	[95% Conf. Interval]
result	68	.3382353	.0573729	.2278938 .463171

-> location = central

-- Binomial Exact

Variable	Obs	Mean	Std. Err.	[95% Conf. Interval]
result	47	.4255319	.0721191	.282579 .5782153

-> location = north

-- Binomial Exact

Variable	Obs	Mean	Std. Err.	[95% Conf. Interval]
result	23	.3043478	.0959439	.1321029 .5291917

-> location = far north

-- Binomial Exact --

Variable	Obs	Mean	Std. Err.	[95% Conf. Interval]
result	16	.3125	.1158781	.11017 .5866206

Hyenas:

. bysort age: ci result if species==2, b

```
-------------------------------------------------------------------------------
-> age = juvenile
-- Binomial Exact --
    Variable |        Obs        Mean    Std. Err.       [95% Conf.
Interval]
-------------+-------------------------------------------------------------
      result |          1           0           0               0
.975*

(*) one-sided, 97.5% confidence interval

-------------------------------------------------------------------------------
-> age = sub-adult
-- Binomial Exact --
    Variable |        Obs        Mean    Std. Err.       [95% Conf.
Interval]
-------------+-------------------------------------------------------------
      result |         11    .7272727    .1342816        .3902574
.9397823

-------------------------------------------------------------------------------
-> age = adult
-- Binomial Exact
    Variable |        Obs        Mean    Std. Err.       [95% Conf.
Interval]
-------------+-------------------------------------------------------------
      result |         24    .7916667    .0828982        .5784872
.9286814
```

. bysort sex: ci result if species==2, b

```
-------------------------------------------------------------------------------
-> sex = male
-- Binomial Exact
    Variable |        Obs        Mean    Std. Err.       [95% Conf.
Interval]
-------------+-------------------------------------------------------------
      result |         17    .5882353    .1193646        .3292472
.815563
```

```
-> sex = female
-- Binomial Exact --
    Variable |        Obs        Mean    Std. Err.       [95% Conf.
Interval]
-------------+-------------------------------------------------------------
      result |         19    .8947368    .0704059        .6686233
.9869878
```

Lions:

```
. bysort age: ci result if species==1, b

-> age = cub
-- Binomial Exact --
    Variable |        Obs        Mean    Std. Err.       [95% Conf.
Interval]
-------------+---------------------------------------------------------
      result |          1           0           0               0
.975*

(*) one-sided, 97.5% confidence interval

-------------------------------------------------------------------------
-> age = juvenile
-- Binomial Exact
    Variable |        Obs        Mean    Std. Err.       [95% Conf.
Interval]
-------------+---------------------------------------------------------
      result |         21    .6190476    .1059712        .3843544
.8189284

-------------------------------------------------------------------------
-> age = sub-adult
-- Binomial Exact
    Variable |        Obs        Mean    Std. Err.       [95% Conf.
Interval]
-------------+---------------------------------------------------------
      result |         26    .3846154    .0954113        .2022602
.5942925

-------------------------------------------------------------------------
-> age = adult
-- Binomial Exact --
    Variable |        Obs        Mean    Std. Err.       [95% Conf.
Interval]
-------------+---------------------------------------------------------
      result |        119    .3277311    .0430286         .244491
.4197786

. bysort sex: ci result if species==1, b

-------------------------------------------------------------------------
-> sex = male
-- Binomial Exact
    Variable |        Obs        Mean    Std. Err.       [95% Conf.
Interval]
-------------+---------------------------------------------------------
      result |         74    .4054054    .0570741        .2927255
.525898

-------------------------------------------------------------------------

-> sex = female
-- Binomial Exact
    Variable |        Obs        Mean    Std. Err.       [95% Conf.
Interval]
-------------+---------------------------------------------------------
      result |         99    .3434343    .0477247        .2508819
.4456307
```

```
age:
           1 cub
           2 juvenile
           3 sub-adult
           4 adult
sex:
           1 male
           2 female
location:
           1 south
           2 central
           3 north
           4 far north


. xi: logistic result i.age i.sex i.location if species==1
i.age             _Iage_1-4           (naturally coded; _Iage_2 omitted)
i.sex             _Isex_1-2           (naturally coded; _Isex_1 omitted)
i.location        _Ilocation_1-5      (naturally coded; _Ilocation_2
omitted)

Logistic regression                               Number of obs    =
138
                                                  LR chi2(6)       =
16.52
                                                  Prob > chi2      =
0.0112
Log likelihood = -84.109501                       Pseudo R2        =
0.0894

------------------------------------------------------------------------------
      result | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf.
Interval]
-------------+----------------------------------------------------------------
     _Iage_3 |    .554393   .3696933    -0.88   0.376     .1500375
2.048499
     _Iage_4 |   .1994199   .1119111    -2.87   0.004     .0663878
.59903
     _Isex_2 |   .6836091   .2575831    -1.01   0.313      .326645
1.430671
_Ilocation_1 |    .392835   .1729385    -2.12   0.034     .1657616
.9309712
_Ilocation_3 |   .2024927   .1280071    -2.53   0.012     .0586566
.6990402
_Ilocation_4 |   .2275533   .1690014    -1.99   0.046     .0530768
.9755764
------------------------------------------------------------------------------

. lfit, g(10)

Logistic model for result, goodness-of-fit test

       number of observations =       138
             number of groups =         9
      Hosmer-Lemeshow chi2(7) =      1.06
                  Prob > chi2 =    0.9937
```

. tab location age if species==1, r ex

location	age: juvenile	sub-adult	adult	Total
south	8	9	47	64
	12.50	14.06	73.44	100.00
central	2	4	31	37
	5.41	10.81	83.78	100.00
north	7	4	12	23
	30.43	17.39	52.17	100.00
far north	3	3	8	14
	21.43	21.43	57.14	100.00
Total	20	20	98	138
	14.49	14.49	71.01	100.00

Fisher's exact = 0.098

Wild dogs:

. bysort age: ci result if species==3, b

```
-> age = juvenile
-- Binomial Exact --
    Variable |        Obs        Mean    Std. Err.       [95% Conf.
Interval]
-------------+---------------------------------------------------------
      result |          8        .875    .1169268        .4734903
.9968403

-------------------------------------------------------------------------------

-> age = adult
-- Binomial Exact --
    Variable |        Obs        Mean    Std. Err.       [95% Conf.
Interval]
-------------+---------------------------------------------------------
      result |         15    .7333333    .1141798        .4489968
.9221285

-------------------------------------------------------------------------------
```

. bysort sex: ci result if species==3, b

```
-> sex = male
-- Binomial Exact --
    Variable |        Obs        Mean    Std. Err.       [95% Conf.
Interval]
-------------+---------------------------------------------------------
      result |         14    .8571429     .093522        .5718708
.9822055

-------------------------------------------------------------------------------

-> sex = female
-- Binomial Exact --
    Variable |        Obs        Mean    Std. Err.       [95% Conf.
Interval]
-------------+---------------------------------------------------------
      result |          8        .625    .1711633        .2448632
.9147666

-------------------------------------------------------------------------------
```

```
. xi: logistic result i.age i.sex i.location if species==1
i.age              _Iage_1-4           (naturally coded; _Iage_4 omitted)
i.sex              _Isex_1-2           (naturally coded; _Isex_2 omitted)
i.location         _Ilocation_1-5      (naturally coded; _Ilocation_2
omitted)

Logistic regression                                    Number of obs    =
138
                                                       LR chi2(6)       =
16.52
                                                       Prob > chi2      =
0.0112
Log likelihood = -84.109501                            Pseudo R2        =
0.0894

------------------------------------------------------------------------------
      result | Odds Ratio   Std. Err.      z    P>|z|     [95% Conf.
Interval]
-------------+----------------------------------------------------------------
     _Iage_2 |   5.014544   2.814078     2.87   0.004     1.669366
15.063
     _Iage_3 |   2.780028   1.467314     1.94   0.053     .9880535
7.822003
     _Isex_1 |   1.462824   .5511906     1.01   0.313      .698973
3.061428
_Ilocation_1 |    .392835   .1729385    -2.12   0.034     .1657616
.9309712
_Ilocation_3 |   .2024927   .1280071    -2.53   0.012     .0586566
.6990402
_Ilocation_4 |   .2275533   .1690014    -1.99   0.046     .0530768
.9755764
------------------------------------------------------------------------------
```