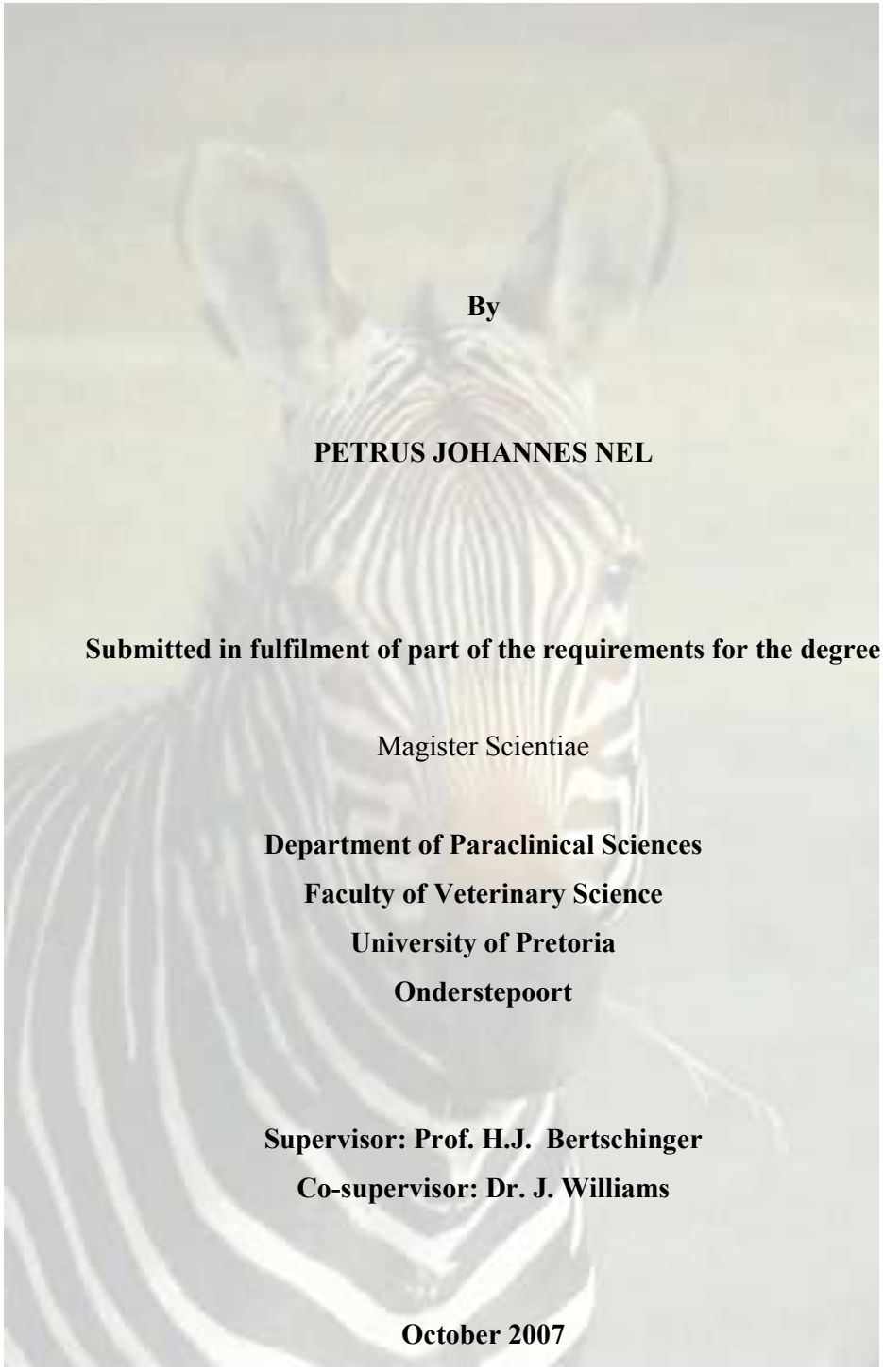
AN OUTBREAK OF EQUINE SARCOID IN A POPULATION OF CAPE MOUNTAIN ZEBRA (*EQUUS ZEBRA ZEBRA*) - A RETROSPECTIVE STUDY



By

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Submitted in fulfilment of part of the requirements for the degree

Magister Scientiae

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October 2007

ACKNOWLEDGEMENTS

It is with sincere gratitude that I thank the following people and institutions:

My supervisor and co-supervisor for their continued guidance and support.

Professor Alan Guthrie for taking initial interest in the study and suggesting the use of the data for post-graduate degree purposes.

The Department of Tourism, Environmental and Economic Affairs of the Free State (DTEEA) for allowing the project, for financial assistance with the disease investigation and for the bursary during the last years of the study.

Personnel from the game capture team of the DTEEA, especially Kees Lawrence and Kobie Clauss for assistance with the capture of the study animals.

Personnel at Gariep Nature Reserve for their support and assistance with the project.

Savvas Vrahimis, Johan Avenant and Ras Venter for comments and suggestions.

Pilots of the helicopters with which the capture was done through the years: Paul Davis, Wayne Grobler, Martin Jacobs, Thilo Kaiser and John Bassi. Thanks for having flown me safely and with such excellent technical capability and professionalism.

My lovely wife Michéle and the two stars in my life, my daughters Alexa and Megan who had to endure my absence during field trips and my “absence” while working on the dissertation.

My Creator most of all, for every day of grace and life.

DECLARATION

I, P.J. Nel hereby declare that the research presented in this dissertation, was conceived and executed by myself, and apart from the normal guidance from my supervisor, I have received no assistance.

Neither the substance, nor any part of this dissertation has been submitted in the past, or is to be submitted for a degree at this university or any other university.

This dissertation is presented in fulfilment of the requirements for the degree Magister Scientiae in Paraclinical Veterinary Sciences.

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Date: 22 October 2007

P.J. Nel

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LIST OF ABBREVIATIONS

BCG	Bacillus Calmette-Guérin vaccine
BNP	Bontebok National Park
BP	Base pairs
BPV	Bovine papillomavirus
BPVs	Bovine papillomaviruses
CITES	Convention on the International Trade in Endangered Species
CMZ	Cape mountain zebra (*Equus zebra zebra*)
DNA	Deoxyribonucleic acid
FS DTEEA	Free State Department of Tourism, Environmental and Economic Affairs
ELA	Equine leucocyte antigen
GMNR	Gamka Mountain Nature Reserve
GNR	Gariep Nature Reserve
HMZ	Hartmann's mountain zebra
HPV	Human papillomavirus
IUCN	International Union for the Conservation of Nature
KNR	Kamanassie Nature Reserve
MHC	Major histocompatibility complex
MZNP	Mountain Zebra National Park
NK	"Natural killer" cells
PCR	Polymerase chain reaction
PVA	Population viability analysis
UK	United Kingdom
USA	United States of America
WHO	World Health Organization

ABSTRACT

Equine sarcoid was diagnosed in the Cape mountain zebra (CMZ) population at the Gariep Nature Reserve (GNR) located in the southern Free State Province of South Africa in 1996. The course of the disease outbreak over the period from 1996 to 2003 is retrospectively described from data gathered during that time. In total, data from 39 affected animals was gathered during the study period. The average population size during the outbreak was 69 individuals. The initial prevalence was 9.4% in 1996. When compared to the neighbouring domestic horse population, where no cases of equine sarcoid had been noted, the CMZ population showed a high prevalence of sarcoid for reasons unknown at the time.

To mimic dynamics in a natural ecosystem with predators, it was decided to remove sarcoid-affected zebra from the population during 1996 and 1997. No sarcoid cases were seen in 1998 and 1999. After thoroughly examining the population in 2000, seven new sarcoid cases were found. Given the endangered status of the CMZ, no further affected animals were culled and a decision was made to study the disease more intensively, with emphasis on epidemiology, aetiology, clinical appearance and pathology, and treatment options as well as to investigate the genetic status of the population and the possibility of a genetic predisposition to the development of equine sarcoid.

Prevalence of sarcoid cases in the Gariep CMZ population increased to 24.7% in 2002. Incidence varied between 4.65-17.6% during the study period with higher incidence rates recorded towards the second half of the study period. No sexual predilection was established. Sarcoids were not seen in animals younger than three years of age. Of the affected individuals, 64.1% had a single lesion and no animal had more than four lesions. Sarcoids were mostly of the fibroblastic (57%), verrucose (16%), and nodular (10%) types or a combination of these. The majority of lesions in males occurred in the inguinal area (55.17%), whereas the majority of lesions in females occurred on the head and neck (41.38%). Because treatment trials were conducted in a number of affected individuals, there were not many untreated control animals in which to study the rate of growth of the tumours, but the average annual increase in lesion size in untreated animals was found to be as much as 260%, becoming so large as to mechanically impede movement. During the study period, known sarcoid-related mortalities numbered four, while nine animals were euthanased for humane

reasons, and ten other animals having been identified once with sarcoid were not seen again and presumed dead.

CHAPTER 1

INTRODUCTION

The Cape mountain zebra (*Equus zebra zebra*) is described as a sub-species of *Equus zebra*; the mountain zebra[52]. With an estimated population of 1600 individuals in 2002, the Cape mountain zebra (CMZ) is currently regarded as one of the most endangered large mammals in South Africa [56] as cited in [52,64,65]. The conservation status of the CMZ is listed as vulnerable in the red data book of the mammals of South Africa[24]. The Convention on International Trade in Endangered Species (CITES) has listed the CMZ on the Appendix I list[29.]

All the South African CMZ descend from only 30 individuals that survived into the first half of the twentieth century. At that stage the species was fragmented into three populations confined to the Mountain Zebra National Park (MZNP), Kamanassie Nature Reserve (KNR) and Gamka Mountain Nature Reserve (GMNR)[8,64]. Apart from one male that was translocated from KNR to MZNP in 1970, there was no gene flow between these populations[77]. Most of the CMZ which have subsequently been translocated to other conservation areas in South Africa originate from MZNP where the population is currently maintained at between 300 and 400 animals. The MZNP produces approximately 40 animals annually for relocation to other conservation areas[56.] Poor reproductive performance in the other two founder populations have prevented them from contributing much to the current number of CMZ in South Africa. The reasons for this poor performance could be habitat-related or from inbreeding depression[77,96].

The CMZ population at Gariep Nature Reserve (GNR) originated from a founder population of seven animals introduced from the MZNP in 1985. One animal died shortly after introduction. In 1987, five more animals were introduced and in 1997 another five, all from MZNP, to improve genetic variability and to induce gene flow artificially between the large founder population at MZNP and this fragmented population at GNR (FS DTEEA: unpublished internal records).

Equine sarcoid was first described as a disease entity in 1936[30]. It has subsequently been diagnosed in all the domestic equids[69]. Olson and Cook[58] suggested that BPV might be the

aetiological agent involved in the development of equine sarcoid after experimental inoculation with bovine papillomavirus (BPV) containing extracts.

Sarcoids were first observed in the CMZ of GNR in June 1995 when nature conservators reported “large growths” on some animals.

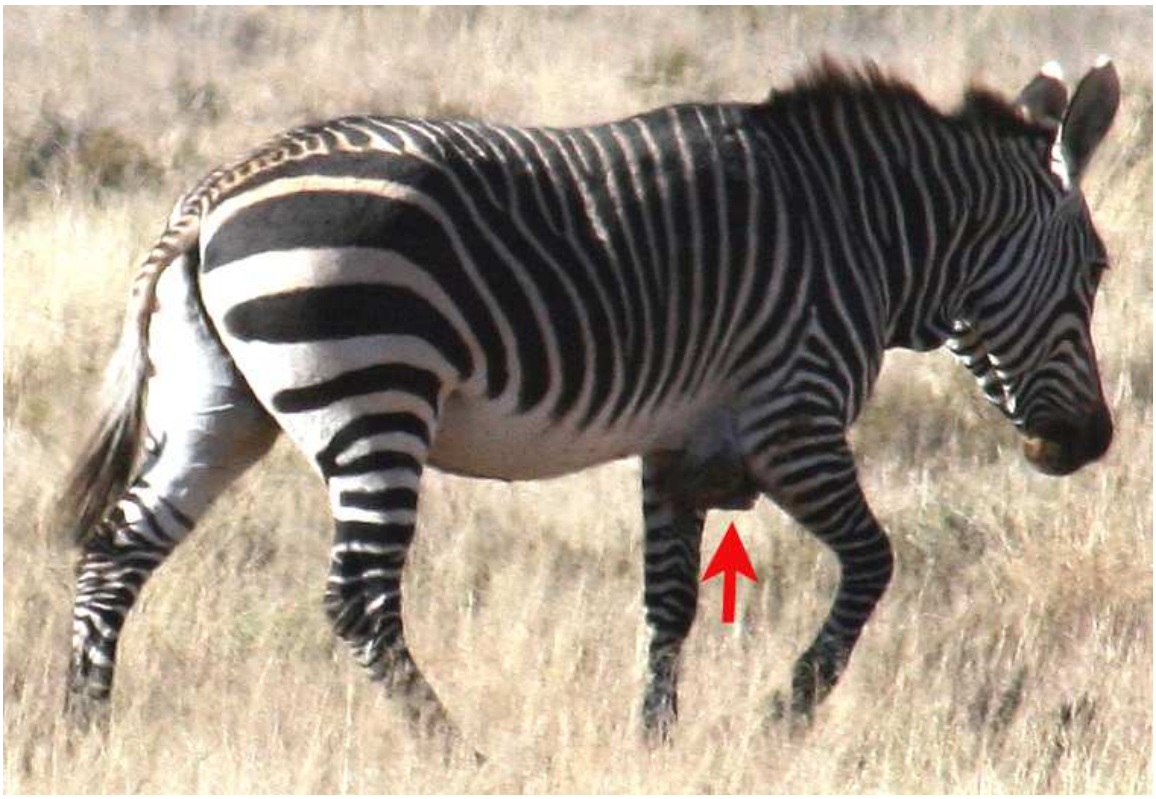

Figure 1: Example of a sarcoid tumour in the right axilla of a CMZ

In January 1996, tumours from two animals were sampled and a histological diagnosis of equine sarcoid was confirmed [Pathology Section of Onderstepoort Veterinary Institute]. Tumour samples were also submitted for electron microscopic detection of BPV viral particles but none were found. A polymerase chain reaction (PCR) test for BPV was not yet available in South Africa. At the time no serious concerns were expressed, as the disease was known to be the most common skin neoplasm in domestic equids. In April 1996, most of the zebra in the population were observed with binoculars to determine the prevalence of sarcoid in the population. When four more zebras were seen with tumours, they were captured and tissue samples collected in 10% formalin. On histopathology all were diagnosed as equine sarcoid.

Although sarcoids have been described in detail in horses and other equids, there is no information on the disease in zebra. Furthermore, the epidemiological aspects of the disease have never been described in free-ranging zebra. Although one could extrapolate from research done on domestic horses, the course of an outbreak of equine sarcoid in free-ranging CMZ could not be determined without species-specific information on aetiology, susceptibility, transmission and many other variables. Where the approach in a domestic horse would be the salvage of the individual for performance or breeding, the approach in wildlife is directed towards the restoration of the health of the population to such an extent that it can grow or maintain itself in a sustainable manner without constant human intervention. Apart from the conservation ethics, treatment of individual clinical cases in wildlife is often difficult from a logistic, husbandry, financial and practical point of view. Most interventions in affected zebra require immobilization and generally from a helicopter. Where follow-up treatment or monitoring is necessary, the animal has to be kept in a specially designed holding boma. Isolation is stressful for herd animals kept in holding bomas and this often results in animals sustaining injuries, even if they are tranquillized. Once the animal has been placed in the holding boma, general anaesthesia is necessary for every clinical examination, surgical procedure or treatment, excepting for low volume injections that can be administered using a pole syringe or drop-out dart.

The initial approach in the GNR was to eliminate all the affected animals from the population in order to prevent the possible "contamination" of the population with genes that might make the population more susceptible to the development of equine sarcoid. The culling of almost 11% of a population of an endangered species is not a decision to be taken lightly, especially in the absence of specific proof of the genetic susceptibility theory. This meant that a decision on how to approach the outbreak of equine sarcoid in the GNR CMZ population had to be made. Accordingly it was decided to investigate the sarcoid outbreak more intensively, especially the course of the disease with emphasis on basic epidemiological parameters. In addition treatment options were to be evaluated. Prevalence was well beyond what would be expected for sarcoid in other domestic equids[35,45,69,76]. With evidence of breed predilection in horses[2,45], the possibility of a genetic aspect to the susceptibility of the Gariep CMZ was contemplated.

The aim of this retrospective study was to analyse the epidemiological data gathered from 1995 to 2003 on the outbreak of sarcoid in the GNR CMZ and to describe some of the clinical and pathological findings of the sarcoid lesions.

CHAPTER 2

LITERATURE REVIEW

2.1 THE GARIEP NATURE RESERVE

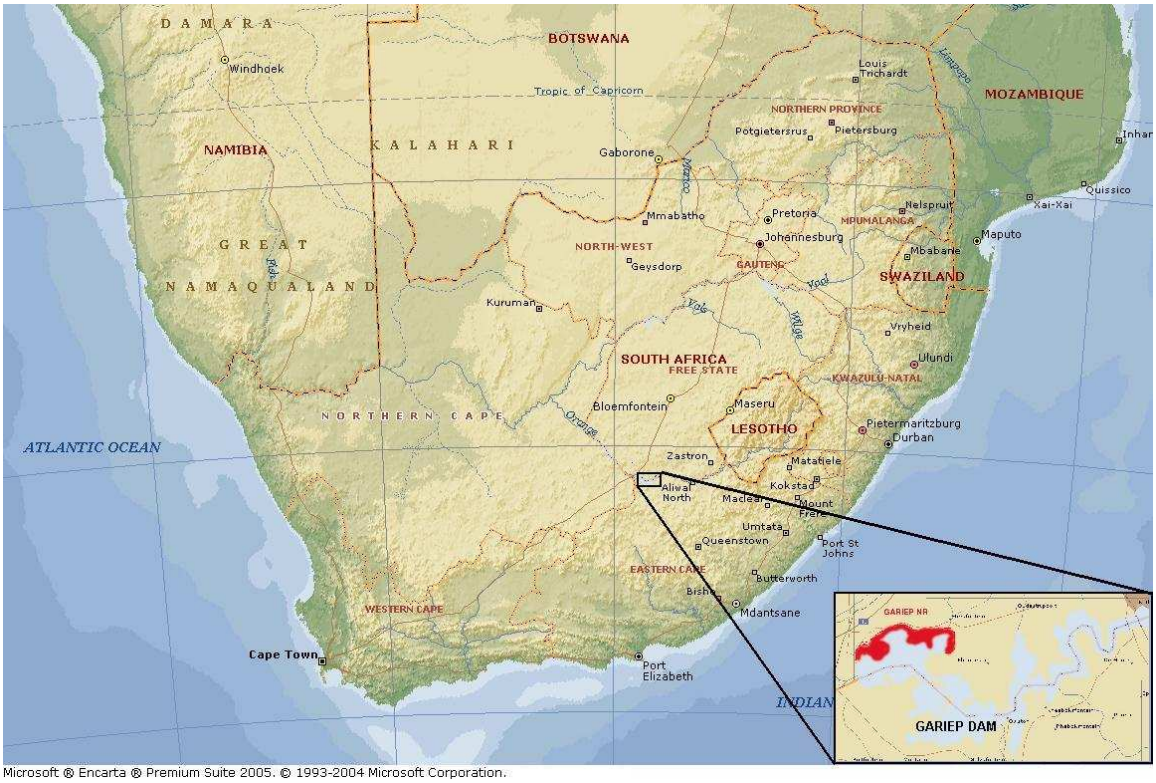

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FIGURE 2: Map showing the location of GNR in South Africa

The GNR is located in South Africa's Free State Province. The reserve borders the northern shore of the Gariep Dam, which was constructed across the flow of the Orange River that forms the southern border of the province. The size of the game-fenced area available to the CMZ population is approximately 6000 ha. It consists of steep rocky, flat-topped hills and undulating plains. The geological formations consist mainly of the Beaufort group sandstone and shale, which is shaped by dolerite dykes and sills. The vegetation is classified as eastern mixed Nama Karoo[41]. The average annual rainfall is 350 mm (range 150–500 mm) and temperatures vary from a minimum of –10 °C in winter to a maximum of 42 °C in summer. The northern side of the GNR borders commercial farmland. Because of the dry climate, agriculture is limited to stock farming (with sheep, goats, cattle and horses) except for some irrigation projects along the Orange River.

2.2 CAPE MOUNTAIN ZEBRA

Cape mountain zebras belong to the genus *Equus* that is the only remaining genus of the Equidae. The *Equus* genus is divided into three sub-genera: *Equus* which includes the true horses, *Asinus* containing the asses and hemiones and the *Hippotigris* sub-genus which includes the zebras. The zebras consist of four species: the Cape mountain zebra (*Equus zebra)*, the Hartmann's mountain zebra (*Equus hartmannae*), the plains zebra (*Equus quagga)* which is divided into six sub-species, and the monotypic Grevyi's zebra (*Equus grevyi)* from East Africa. The CMZ is found in the western- and eastern Cape provinces of South Africa while the Hartmann's mountain zebra (HMZ) is found in the north-western part of the northern Cape province of South Africa, coastal Namibia and southern Angola[27]. Although *Equus zebra* and *Equus hartmannae* are now considered different species, they were until recently still considered to consist of two sub-species: *Equus zebra zebra* (CMZ) and *Equus zebra hartmannae* (HMZ) and distinction as different species still remains controversial[52,56,77,83].

Cape mountain zebras are amongst the most endangered of the larger South African mammals. By 1950, the total number of CMZ in South Africa reached an alarmingly low number of approximately 100-150 animals. The estimated number in 2002 was 542 animals in national parks, 491 in provincial nature reserves and 165 in other reserves and on game farms[56]. At a constant annual growth rate of 9.5%[56] the estimated total number in 2006 was about 1700 animals. In contrast, the number of HMZ in Namibia was estimated at 25 000 animals in 2002[56]. Given the number of CMZ in 1956 (150 animals)[56] and the estimated number in 2001 (1200 animals), the annual population growth rate for that entire period was only 5%. The GMNR population grew at only 3% per annum from 1974 to 2004, possibly due to marginal habitat and/or inbreeding depression[96]. As more breeding herds were established in better habitats, production increased and was calculated at 8.6% for the 10-year period 1985-1995 and even higher at 9.6% for the period 1995-1998[56]. Perhaps the increased production rate of the meta-population reflects an increase in the proportion of CMZ in favourable habitats compared to poorly performing ones in marginal habitats.

The three most important risk factors facing small isolated populations are environmental conditions, stochastic events and reduction in genetic variation. Genetic variation can be lost due to genetic drift and an increased probability of breeding with close relatives[11]. Fillies in a

free-ranging population of CMZ usually leave the breeding herds at the average age of 18 months, which is before the first fertile oestrus. Colts leave the natal herd at an average age of 24 months to join other related males in a non-breeding herd. Both the colts and fillies leave their natal herds of their own accord and not because of aggression from other herd members. A herd stallion will often try to prevent sub-adults from leaving his herd. Fillies are either taken up into other existing breeding herds (in the absence of males of reproductive age in non-breeding groups) as subordinate females, or they join a non-breeding herd of unfamiliar males, later to form a breeding-herd with one of these unrelated stallions[65]. Stripe-pattern recognition could be a mechanism to prevent incest-mating[72]. These natural behavioural traits would thus normally prevent inbreeding in a large population in a large area. In smaller populations, this mechanism may not be as effective. In general, colts join bachelor herds that are the male reservoir of a population, competing for the establishment of new breeding herds. Colts have however been observed to establish themselves as the breeding stallion in their maternal herd[64]. The only way in which a filly will form a new breeding herd with her own brother is if the brother left the natal herd before the filly was born, in which case he would not be familiar to her, and therefore would not be avoided[65,72].

A study in plains zebra has established that small populations (<100 individuals) isolated for a long period (>20 years or more) and which started with a low founder population (<10 individuals) displayed a 19% loss of polymorphisms compared to the original population. Heterozygosity did however not decrease as expected from studies in ungulates. With larger founder groups (>20) up to 90% of genetic variation can be maintained if population sizes of 200 individuals can be attained. If a small population of <20 animals is isolated, the probability of extinction due to loss of genetic variation in the next 200 years is very real. To restrict loss of heterozygosity to 10% over 100 years in a small population of ±20 animals, a harem group of five animals needs to be introduced every five years. A harem group could consist typically of a breeding stallion, three females and a foal[11]. The interval can be increased with increasing population size (15 years for a population of 120 animals).

CMZ populations are organized into breeding herds that consist of a single adult stallion, one to five mares and their offspring (herd usually not more than 13 in total). The mean size of breeding herds in a study of CMZ in the MZNP was 4.7 animals per herd with a mean of 2.4 breeding females per herd. Breeding herds remain stable for many years and are usually only disrupted when the stallion is displaced because of poor condition, death or removal. Females

then join other breeding herds or the herd is taken over by another stallion. The herd stallion is at the top of the herd hierarchy. The bond between mares is also important in herd cohesion after displacement of the herd stallion. Bachelor herds are not as cohesive and stable as breeding herds. They comprise 17-20% of the population. There is no social hierarchy in bachelor herds[65].

2.3 SARCOIDS

2.3.1 OVERVIEW

Sarcoids were first described and characterized as a clinical entity in horses by Jackson in 1936[30]. Sarcoid has been reported worldwide in horses, donkeys and mules[30,45,62,70,85]. According to Miller and Campbell[51], equine sarcoid was the most common skin neoplasm in every region surveyed in the Queensland area of Australia. Sarcoid was also found to be the most common skin tumour in horses, mules and donkeys in general[45]. The World Health Organization (WHO) Tumour Classification System lists the sarcoid as a benign tumour[35]. Although the tumour can be locally invasive, it is limited strictly to the skin and directly underlying tissues and does not spread to internal organs[35]. Sarcoids can be defined as locally aggressive fibroblastic benign tumours of equine skin[70]. They can occur as single or multiple lesions in different forms, ranging from small wart-like lesions to large ulcerated fibrous growths. Lesions can occur at any site on the body but sites of predilection are in particular the paragenital region, the thorax, abdomen and head. Sarcoids frequently occur at sites of previous injury and scarring[70]. Histologically, the equine sarcoid can be classified as a fibroma or fibrosarcoma[20]. In cases where there is epithelial proliferation, the term fibropapilloma is used[77].

2.3.2 AETIOLOGY

There is considerable evidence that equine sarcoid is caused by a virus closely related to or identical to bovine papillomavirus[2,9,17,44,45,61,75]. Olson and Cook as cited in[45] demonstrated in 1951 that intra-dermal inoculation of cell-free extracts from bovine skin tumours containing bovine papillomavirus into horses, caused lesions resembling equine sarcoid. This was the first suggestion that BPV may be responsible for equine sarcoid. Equine cutaneous papillomavirus is however not related to or involved in the aetiology of equine sarcoid.

Neither plain electron microscopy, nor serum antibody tests were of value in demonstrating BPV as the cause of sarcoid[45]. Molecular techniques were used to substantiate the involvement of BPV in equine sarcoid. The BPV-DNA is however not integrated into the host cells in equine sarcoid[37] as cited in [45,92].

BPV is a small DNA virus of the family Papovaviridae. The six subtypes are divided into two groups. Viruses in Subgroup A can transform fibroblasts and epithelial cells. Viruses in Subgroup B can only transform epithelial cells[19]. The Papillomaviridae is a large family of viruses that normally infect epithelial cells causing hyperproliferative lesions known as warts, papillomas or condylomas in animals and humans[20]. Typically, papillomavirus-induced lesions are benign, self-limiting and usually regress spontaneously. Papillomaviruses have been associated with fibropapillomas in cattle[18], equids[3,19,57,61,68,75,92], sheep[26], cervids[36], camelids[80] and in cats[79] and water buffalo[60]. Some camelids have also recently been diagnosed with mucocutaneous fibropapillomas that are histologically similar to equine sarcoid and contain papillomavirus[80].

The equine sarcoid can be considered a neoplasm with an infectious origin and a variety of manifestations resulting from interactions between the causative agent, the environment and the host genome. There is currently little doubt that Bovine Papillomaviruses Sub-types 1 and 2 or equine-adapted minor variants of these viruses are responsible for sarcoid development[25].

It has been found that MC-1 cells (an equine sarcoid-derived cell line) produce a retrovirus *in vitro* which resulted in tumour growth when injected into nude mice[22]. Normal equine dermal cells contain a repressed pro-virus homologous to the MC-1 viral genome which is spontaneously expressed in sarcoid cells. This MC-1 retrovirus has however been found to be non-oncogenic and replication defective. The role of this MC-1 retrovirus in the epidemiology of equine sarcoid is unknown[21].

Papillomaviruses are considered to be highly host specific with as many as 60 human, six bovine and various other animal sub-types characterized. Vegetative growth of papillomaviruses only takes place in the upper epithelial strata where they can be demonstrated by *in situ* hybridisation to detect the specific DNA, by immunohistochemical detection of papillomavirus structural antigens and by electron microscopic detection of

intranuclear virus particles. Papillomaviral antigen has been detected in both of two cutaneous papillomas[82].

BPV plays a central role in equine sarcoid. In a study of 58 histologically confirmed sarcoids in horses, DNA for BPV-1 and BPV-2 could be amplified in each sample. No apparently normal skin from affected horses yielded any BPV-DNA and neither did lymphocytes[61] BPV Types 1 and 2 were also found in sarcoid lesions examined in Germany[87]. A latency of papillomavirus has been found in its natural host, the presence of which has been confirmed in the lymphocytes of cattle[32].

BPV-DNA has been demonstrated in superficial swabs from the skin of affected horses. BPV-DNA could also be detected on the normal skin of four out of nine non-affected horses living in contact with sarcoid-affected horses, as well as on the normal skin of one horse living in contact with a cow with warts and on one horse not having any contact with cattle or other affected horses. BPV was also detected in the environment where sarcoid affected horses were kept[10].

Genomic DNA was extracted from snap-frozen sarcoid tissue from donkeys by digestion of 10 µg of tissue with *Hind*III, *Bam*HI and *Kpn*I restriction endonucleases, doing agarose gel electrophoresis and photography. Southern blotting, hybridisation to full length molecularly cloned BPV-2, BPV-5 and BPV-4 genomes and autoradiography was carried out. They then linearised one of the papilloma viral elements at the unique HindIII site and cloned it into plasmid pIC-20H and transfected it into DH5alpha competent *Escherichia coli*. Mini preparations of the amplified plasmid DNA were then subjected to agarose gel electrophoresis. Southern transfer and hybridisation to molecularly cloned BPV-2 was then done. The genomic organization of the clone was then compared to that of BPV-1 and BPV-2. Nucleotide sequences were analysed on alkali-denatured double-stranded DNA templates. The source of template DNA was purified by acid-phenol mini preparation of plasmid DNA. Two primers were used namely M13 and a custom-synthesized 15mer selected from the E5 open reading frame of BPV-1. The sequence was analysed and compared with the published sequences of characterized papillomaviruses. The results from donkey sarcoids in this study showed full length (± 8 Kbp) BPV**,** very similar to BPV-1 and BPV-2[75].

Sarcoids induced experimentally in horses using BPV tend to regress spontaneously and result in production of antibodies to the virus whereas naturally occurring sarcoids seldom regress spontaneously. Antibodies against BPV have not been detected in horses with sarcoid[45].

Analyses of bovine warts revealed at least 6 distinct BPVs that fall into two groups based on histopathology. One group is associated with fibropapillomas and the other with papillomas[32,33]. BPVs found in fibropapillomas (Types 1, 2 and 5) showed varying degrees of DNA sequence homology and are the most commonly found BPV in equine sarcoid[3]. In contrast to the study conducted by Otten *et al*., in 1993[61] where no BPV-DNA was found in normal skin, BPV was demonstrated in normal skin from sarcoid affected horses in 65% of cases examined at the University of California-Davis Veterinary Medical Teaching Hospital. The closer a sample was taken to the sarcoid lesion, the more likely normal skin was to contain BPV-like DNA. Of the positive samples taken from normal skin, 71% of samples within 3-4cm from a sarcoid tumour were positive for BPV-DNA, compared to only 29% in samples taken more that 20cm away from a sarcoid tumour. In 27% of sarcoid affected cases, no BPV-DNA was found in normal skin. Normal skin in non-affected horses was however consistently negative for BPV in this study. In this study, 98% of sarcoid tumours yielded BPV-DN[19,89].

In a Swiss study of horses with sarcoid, BPV-1 was found in 16 of 17 cases whereas BPV-1 and BPV-2 were found in equal numbers in 12 sarcoids from the United States of America (USA). The number of viral genomes varied widely among sarcoid samples (2-800 copies/cell) but no BPV-like DNA was detected in lymphocytes from sarcoid affected horses. Application of blot-transfer hybridisation methodology in conjunction with endonuclease cleavage patterns of viral sequences easily distinguishes different BPV types obtained from fresh sarcoid[3]. In another study, 38% of sarcoids contained only BPV-1 DNA and 55% contained only BPV-2 DNA. Seven percent of sarcoids contained both BPV-1 and BPV-2 DNA[19]. Of 13 CMZ sarcoid lesions sampled at GNR in 2003, seven contained BPV-1, four contained BPV-2 and one lesion contained both BPV I and 2[93].

The occurrence of restricted cleavage patterns, which differ from those predicted from the nucleotide sequences of prototype BPVs suggest the possibility of variants specific to horses arising through mutational events[45]. Some authors doubted the role of BPV in equine sarcoid given the difference in behaviour, gross morphology and histopathology appearance between

lesions induced in horses by injecting BPV and naturally occurring sarcoid lesions. Inability to induce lesions in BPV susceptible calves using sarcoid tissue also supports this view[84].

It seems that contact with the virus alone is insufficient for tumour proliferation: skin trauma, the immunological status and the genetic constitution of the individual horse also appear to play an important role[10]. The virus might also remain in a latent phase in the fibroblasts until other factors trigger cell transformation[19].

2.3.3 MECHANISM OF TUMOUR INDUCTION

The mechanism of tumour induction of BPV in cattle has been described in some detail[20]. Some of the papillomavirus types (including BPV 1 and 2) can infect fibroblasts and induce fibro-epithelial tumours, causing benign fibropapillomas in cattle. BPV 1 and 2 have a genome of 7900 bp with at least nine potential reading frames. The genome can be split into two principal regions namely the early (E) and late (L) regions. The E-region encodes the transforming proteins E5, E6 and E7, and the replication and transcription regulatory proteins E1 and E2. The L-region encodes the structural proteins of the virus, L1 and L2. The two regions are separated by non-transcribed DNA, called the long control region, which contains the transcriptional promoters and enhancers, the origin of DNA replication and binding sites for numerous cellular transcription factors.

Initially, replication of the virus genome is linked strictly to the differentiation state of the infected cell. Basal keratinocytes are infected initially after which E-region genes are expressed in the undifferentiated basal and suprabasal layers. Viral replication takes place in the differentiating spinous and granular layers and expression of the late structural proteins is limited to the terminally differentiated cells of the squamous layer. Here the virus particles are encapsidated and released into the environment as the cells die. Deregulated expression of the early virus genes, which results in an uncontrolled proliferation (and loss of differentiation) of the infected cells leads to the malignant transformation. As the major transforming protein, E5 is localized in the Golgi apparatus and other intracellular membranes. It activates the platelet-derived growth factor receptor (PDGF-R) in transformed cells. The stimulation of the PDGF-R activates a receptor-signalling cascade, resulting in an intracellular growth stimulatory signal. E5 also binds to a component of gap junctions and of the vacuolar ATPase leading to

the down-regulation of gap junction intracellular communication with the consequent isolation of the infected cell from its neighbours. This binding of E5 also eventually leads to intracellular retention of major histocompatibility complex (MHC) Class I molecules.

The absence of MHC Class I from the cell surface would help the infected cells evade host immunosurveillance. E5 also activates numerous kinases, which interferes with proper cell-cycle control and signal transduction cascades. However, cell transformation by E6 appears to be independent of its transcription transactivation function. While it has been shown that human papillomavirus (HPV) E6 binds and stimulates degradation of p53, BPV E6 does not affect p53. Instead, the transformational ability of BPV E6 is linked to its ability to bind and interact with many proteins, transcriptional co-activators and focal adhesion proteins, leading to a disruption of cytoskeleton and vesicular traffic pathways, respectively. The cytoskeleton is important in cellular morphology, motility, division and cell–cell and cell–matrix interactions while the AP-1 complex plays an important role in the control of cell proliferation and differentiation. This is however not necessarily the exact mechanism of tumour transformation in equine sarcoid as there are important differences in the expression of papillomas in bovines and sarcoids in equids[20].

P53 tumour suppressor protein is one of the genes coordinating central cellular defence. By modulating the transcription of genes of several regulatory proteins, p53 can lead to the block of cellular proliferation or induction of apoptosis. It is especially important in the detection and prevention of growth of many tumours. Many tumours in humans as well as fibrosarcoma, lymphosarcoma and solid mammary gland carcinoma in felines have been shown to contain mutant p53[47,48]. Several DNA viruses encode for proteins that bind p53 and counteract its effects on the viral multiplication by modulating p53 activity or in the case of E6 from high risk HPV, can degrade p53 thereby preventing it from blocking cellular proliferation or induction of apoptosis[28]. Where DNA integrity is affected, nuclear p53 rises sharply and “switches off” DNA replication by a specific arrest of the cell cycle in the G1 phase to allow time for DNA repair. Cells containing mutant p53 or lacking p53 are unable to arrest the cell cycle in the G1 phase. Because of mutant p53 having a much longer half-life, the nuclei of tumour cells contain accumulated amounts of p53 that can be detected immunohistochemically[86]. No mutant p53 was detected in any of 25 sarcoid samples from horses examined after DNA extraction and PCR amplification and analysing the amplified sections using agarose gel electrophoresis[46]. Aberrant perinuclear localisation of p53 has

however been demonstrated more recently in 44% of equine sarcoid lesions□44, suggesting that mutational independent inactivation of p53 occurs commonly in sarcoids. The significance of these finding remains to be elucidated. Although no mutations of p53 were found in 28 sarcoid affected horses compared to 11 healthy horses, the functional inactivation of p53 by BPV-encoded E6 protein may be a possibility[15].

An increase in the activity of the oncogene c-myc has been found in sarcoid tumours. The degree of activity of c-myc was associated with the aggressiveness of the sarcoid tumours. Except for over-expression of c-myc, there was also decreased degradation of c-myc in sarcoid tissue, but not in normal tissue surrounding sarcoids. C-myc activation however did not appear to be as a result of viral sequence insertion. The hypothesis is that BPV affects normal cells in two ways:

a) It provides a trigger for tumour formation and
b) It activates the c-myc oncogene, which results in uncontrolled tumour cell growth. It was also found that BPV-derived oncogenic protein (E5) expression correlated with tumour behaviour[89].

BPV-DNA has been demonstrated in biopsies obtained from the normal skin of horses affected with equine sarcoid, which could indicate the occurrence of viral latency[19,44,92]. The virus has however not yet been demonstrated in the normal skin of horses without equine sarcoid[19,61,88]. Latency of the bovine papillomavirus has been demonstrated in the normal skin and in circulating lymphocytes of cattle both in animals with and without clinical signs of papillomatosis[16].

Activation of latent papillomavirus genomes by chronic epidermal irritation has been documented in *Mastomys natalensis*. Irritation resulted in hyperplasia of the epidermis, which led to the amplification of papillomavirus genomes. Certain trans-acting factors are induced by growth factors that are liberated as a result of tissue damage. Growth factors are liberated as part of the wound healing process to initiate tissue regeneration. Trans-acting factors such as serum response factor then binds to the regulatory regions of the papillomavirus, which induces the expression of viral genomes. This explains the tendency for human papillomas to recur at the site of surgical excision. More than 60% of normal tissue samples taken from areas adjacent to human papillomas contained latent HPV, which is then activated by the normal healing process after surgery, leading to new papillomas a few weeks after surgery[81].

Knottenbelt mentioned that sarcoids in horses usually occur in areas of thin skin with sparse hair cover and a tendency to sweat (also areas that biting flies tend to prefer) and that sarcoids in other locations usually develop at sites of skin damage[35]. The possibility therefore exists that for BPV-DNA to be activated, some form of epidermal damage or irritation is necessary for the activation of trans-acting factors which bind to the regulatory regions causing expression of the viral genome which in turn leads to development of sarcoid tumours. This may explain why transmission studies were more successful where BPV extracts were applied topically to scarified skin, compared to intradermal injection[45,59].

2.3.4 PATHOGENESIS

Papillomaviruses can infect species other than that with which they are commonly associated and this can result in a different pathological outcome from that in the normal host. This is seen in cottontail rabbits where cottontail rabbit papillomavirus causes mostly papillomas in the natural host, but it induces carcinomas at a much higher frequency in domestic rabbits. Similarly, BPV has been found to induce benign fibroblastic tumours in certain mice but in hamsters it causes malignant fibroblastic tumours. When HPV causes malignancy, the viral DNA is often integrated into the host genome, which loses regulated expression of the transforming viral genes. In contrast, BPV genes are maintained episomally during the transformation of the host cells. Only the early genes are transcribed and virus capsids are not formed. The reason may be that the expression of capsid proteins requires the cellular environment of the well-differentiated keratinocytes found in the host cell[20].

Although BPV-DNA has been detected widely in equine sarcoid, and mRNA expression for L1 has been shown[54], BPV structural proteins and virus capsid formation is not evident in equine sarcoids[76]. The infection of equine fibroblasts by BPV seems to be non-productive[20]. This observation is supported by the experiment of Ragland and Spencer where experimental inoculation of sarcoid extracts in cattle did not induce warts[68].

Sequence variations within papillomavirus types have recently been demonstrated. This can affect the cellular location and function of oncoproteins that in return may affect the transforming ability of the virus and the pathogenesis of tumour formation[20].

2.3.5 INCUBATION PERIOD

In a case of auto transmission of sarcoid tissue in horses by topically inoculating scarified skin, the incubation period was six months[59]. Voss indicated that incubation period differed between homologous transmission (115 days) and autologous transmission (57 days) in transmission studies conducted by him[95]. Incubation periods of five and ten months have been documented in two donkeys inoculated intradermally with a cell-free sarcoid extract from a horse[70]. Syrian hamsters injected subcutaneously with BPV developed tumours after 25-43 weeks[1]. In an epizootic of equine sarcoid in horses, it was thought that the outbreak started after the introduction of a large number of horses to a herd. In this outbreak, the incubation time was 8-12 months if the assumption that the new batch of horses introduced the aetiological agent was correct[69].

2.3.6 TRANSMISSION

Although BPV Types 1 and 2 have been shown as the primary causative agents of equine sarcoids, there is currently no definitive evidence of the way the disease is transmitted[20]. Transmission studies have been relatively unsuccessful, with only a limited number of sarcoid tissue extracts producing disease when injected into other sites on the sarcoid donor animal or other horses[45]. Olson successfully auto-transmitted sarcoid on a mule by topically inoculating scarified skin with a fresh suspension of pulverized sarcoid tissue. When this method was repeated later on the same animal and on other mules and horses, it failed to result in sarcoid transmission. Boiled material and a fresh suspension injected intradermally and subcutaneously also failed to produce sarcoid[59].

According to Voss, autologous transfer of whole tumour material was much more effective (80%) than homologous transfer (17%)[95]. Inoculation of scarified skin with minced sarcoid suspension as well as with cell free sarcoid suspensions resulted in sarcoid transmission whereas intradermal or subcutaneous injection and topical application onto intact skin were unsuccessful. Ragland et al.,[70] were unsuccessful in producing transmission of sarcoid using the methods described by Voss[95], but they were successful in producing sarcoid in 2 out of 5 donkeys five and ten months following intradermal injection of a cell-free extract of sarcoid from a horse.

BPV inoculated intradermally into horses produced fibrous nodules, which appeared 12-27 days later and regressed spontaneously 100-150 days after initial appearance. One of the animals was inoculated onto scarified skin, which produced a rapidly growing tumour, which recurred three times after excision. On histopathology, this specific tumour was diagnosed as sarcoid[57].

Cattle inoculated with equine sarcoid extract (intradermally and onto scarified skin) showed no response, but they did develop warts when injected with BPV later[71].

There are strong indications that flies are responsible for spread of sarcoid on an individual horse. It is uncertain whether insects are responsible for transmission between horses. Because no viral genomes have been found in the blood of affected horses, spread via the blood stream is unlikely[35,91]. BPV- DNA sequences have been detected in face flies, which are commonly seen around wounds. The same viral DNA sequences were detected in the horses from which the flies were removed[20]. With the presence of BPV-DNA on the surface of fibroblastic sarcoid lesions, on normal skin of affected and ”in-contact” horses as well as in the habitual surroundings of affected horses, the possibility of flies acting as vectors seems viable, even if they act only as mechanical vectors.

Sarcoid free donkeys associated closely with sarcoid affected animals were more at risk of sarcoid development, supporting the viral aetiology and possibility of vector transmission. Donkeys introduced into the population with sarcoid affected donkeys were most likely to develop sarcoid 2-3 years after potential exposure[75].

An outbreak of sarcoid has been described in a horse population, 8-12 months after introduction of a large number of new horses. It was concluded that the newly introduced horses brought the viral agent with them. As no mention is made of contact with BPV infected cattle, one can assume horse-to-horse transmission of BPV[69].

2.3.7 SUSCEPTIBILITY

There seems to be some breed predilection in horses regarding the prevalence of sarcoid. This was determined using admission records of the veterinary hospitals of Ohio and Cornell Universities. The average breed prevalence in relation to admissions was as follows: Thoroughbred - 0.55%, Quarter Horse – 0.89%, Standardbred - 0.26%, Appaloosa - 0.97%, Arabian, 1.36% and other – 0.96% with a total average of 0.7%[45]. No conclusive breed predilection was however found in a Queensland survey[51].

Quarter horses were found to have almost twice the risk of developing sarcoid compared to Thoroughbreds, while Standardbreds had a much lower risk of developing sarcoid relative to all breeds[2]. In a North American study of 503 sarcoid cases (from medical records for 22 Veterinary Teaching Hospitals), Appaloosa, Quarter horse and Arabian breeds had the highest proportional morbidity rate, with Thoroughbreds at a slightly lower rate and Standardbreds significantly lower than all other breeds[53]. It was found that the MHC equine leukocyte antigen (ELA) B1 occurred more frequently in sarcoid affected horses than in unaffected horses[39]. The MHC antigen ELA B1 was found to be rare in Standardbreds[50]. This might explain the lower frequency of sarcoid in this breed. One should however be careful in the interpretation of sarcoid risk data for different breeds and not confuse increased risk with increased susceptibility. The fact that Appaloosa and Quarter horse breeds are more closely associated with cattle on the ranches where they are used as working horses may explain their higher risk profile compared to Thoroughbreds (stud and training) that are seldom in close contact with cattle, thereby decreasing the risk of infection with BPV[53]. ELA haplotypes A3 and W13 appeared more frequently in sarcoid affected horses than in sarcoid-free animals and 40% of sarcoid prevalence in Swedish halfbreds was found to be associated with the genetic background of the affected individual[13].

The ELA association with equine sarcoid demonstrated in population and family studies is one of the strongest MHC disease associations described in any species. The precise MHC genes responsible for sarcoid susceptibility have not yet been identified[45]. A strong correlation was found in Thoroughbreds between sarcoid susceptibility and MHC encoded antigens W3 and B1[50].

The MHC is comprised of different classes of genes with different locations and functions. The Class II genes have been implicated in sarcoid susceptibility. This class codes for proteins bound to the surface of lymphocytes and macrophages, which act as receptors for foreign

antigens. They also present these antigens to the cells of the immune system. The presence of the A5 haplotype has been associated with early onset (2.9 ±1.5 years) of equine sarcoid while the W13 haplotype has been associated with increased risk of recurrence[66].

Class I MHC antigens are involved in the elimination of tumour cells[39] Cytotoxicity of natural killer (NK) cells has been found to function sub-optimally in horses carrying W3 and B1 haplotypes or depending on breed, the W5 and W11 haplotypes. The fact that ELA W5 and W11 (depending on breed) are associated with sarcoid occurrence, means that these ELA Class I antigens are in a breed-dependent disequilibrium with genes (Class II MHC genes) that are more closely associated with sarcoid pathogenesis. If this is the case, then ELA Class I antigens can serve as markers for the genes associated with the pathogenesis of sarcoid susceptibility. It has been suggested that horses carrying certain haplotypes, as markers for sarcoid susceptibility, should not be used for breeding (or sarcoid affected sires for that matter) in order to reduce the prevalence of sarcoid in the specific breed or line. It is also possible that the Class I antigens detected as more prevalent in sarcoid free horses, may be directly responsible for the elimination of sarcoid tumour cells[13]. In the face of increased susceptibility to sarcoid in different breeds the conclusion is that the A and DQß loci may only act as markers and not be actively involved in the pathogenesis of the disease[40]. The fact that peripheral lymphocytes in sarcoid affected horses show a slight increase in cytotoxicity against sarcoid derived cell culture lines, also supports the suggestion that Class I ELAs are involved in the elimination of sarcoid cells[13].

When BPV was inoculated intradermally into young ponies, many developed dermal fibrosis distinguishable from sarcoid by the absence of the typical epidermal component. Some animals displayed more extensive papillomatous and ulcerated growth. Spontaneous regression occurred in some of the ponies but in others the tumours that persisted, resembled equine sarcoid after 6 months. All these lesions had however regressed at one year, which is uncommon for sarcoid. This may indicate that there is a difference in susceptibility between individuals[67]. In an epizootic of sarcoid in horses in the USA, four of the five affected animals were members of a highly inbred family[69].

Until more is known about the genetics of the disease, it is not possible to predict whether an individual is susceptible to sarcoid development or not[35].

2.3.8 IMMUNE RESPONSE

Lymphocytes cultured from horses affected by sarcoid showed an increased proliferative response when exposed to cells of the Mc-1 cell line (sarcoid cell culture) compared to normal allogenic fibroblasts[13,97]. They also showed an increase in cytotoxicity toward Mc-1 cells compared to lymphocytes from unaffected horses. In contrast with Mc-1 cells, freshly excised sarcoid tissue does not express large amounts (if any) of membrane-associated antigens, which may represent viral structural elements or virus-induced neo-antigens[12]. Tumour-specific transplantation antigens have been detected in the Mc-1 cell line[97]. There seems to be histological evidence supporting a cell-mediated immune response in regressing sarcoids[59] although sarcoids rarely regress spontaneously[23]. No significant changes were found in CD4+, CD8+ or CD21+ lymphocyte sub-populations after repeated auto-vaccination. This makes it difficult to explain the immunological functioning of auto-vaccines[55].

When Bacillus Calmette-Guérin (BCG) vaccine is injected intra-lesional into sarcoid, muramyl dipeptide (a structural component of the mycobacterial cell wall) is responsible for the immunostimulation, causing a delayed hypersensitivity reaction to the sarcoid tissue into which it is injected. The tumour is then destroyed by macrophages that generate a proteolytic activity and synthesize cytotoxic oxygen-derived free radicals, and also by cytotoxic lymphokines produced by sensitised T-lymphocytes and NK cells[23].

In most cases of papillomavirus infection, the lesion regresses spontaneously following activation of the host immune response. Papillomavirus has the ability to subvert the host immune response by the collective effects on the interferon pathway, antigen presentation, interleukin-18 inhibition and down-regulation of MHC I. In addition, the limitation of the viral replication cycle to the epithelium, as well as low-level expression of the virus proteins and an absence of inflammation, leads to minimal exposure of papillomavirus to immune cells[57]. The fact that sarcoids do not regress spontaneously, may suggest that similar immuno-evasive mechanisms may be used by BPV to persist and lead to the invasive growth of fibroblastic cells in sarcoid tissue[20]. Down-regulation of MHC Class 1 expression by E5 oncoprotein of BPV has been demonstrated[4] and may affect the detection of infected cells by cytotoxic T lymphocytes. MHC Class 1 antigens are retained in the Golgi apparatus of the cell because of E5 protein expression and thus prevented from being transported to the cell surface. In this manner, viral peptides escape the immune system preventing regression of

sarcoid lesions. It was shown that this inhibition of MHC I is not irreversible, as beta and gamma interferon treatment increases MHC I synthesis (but not transport to cell surfaces)[43]. Very little is however known about the immune response to equine sarcoids[20].

2.3.9 EPIDEMIOLOGY

Climate

In horses sarcoid was found to occur with equal frequency in the tropical north and sub-tropical south of Queensland in Australia[51]. In cooler Northern climates, sarcoids tend to occur predominantly on the head and abdomen of horses, whereas in warmer climates, they tend to occur mainly on the limbs[23].

Seasonality

In the Queensland survey, sarcoids occurred equally throughout the year. It is however difficult to determine if transmission is seasonal, especially given the highly variable latent period before growths become visible[51]. Sarcoids are commonly reported to grow during winter whereas in summer they tend to multiply on individuals. Flies may be responsible for the latter observation[35].

Age at onset

Different age groups were almost equally susceptible to the development of sarcoid, with the one- to six-year-old group being the most common age at which sarcoids were diagnosed[51]. The prevalence was found to increase with advancing age to a maximum of 20% in six-year-olds followed by a subsequent decrease to reach a minimum in 8-year-olds. Thereafter case frequency increased slowly with age (6% of animals > 15 years), but not nearly as high as in younger animals[91]. In a population of donkeys in the United Kingdom (UK), the peak prevalence was found in animals four-to-five years old with two-to-five year old donkeys (prevalence 15.6%-25%) being the most frequently affected group[75]. In another study, the risk of sarcoid development in horses increased gradually up to the age of 15 years old[53]. In different studies in domestic equids, the earliest ages at onset were one year[45], 6 months[69] and one month old, respectively[84]. In conclusion, it seems that equids of any age are susceptible to the development of sarcoid.

Sexual predilection

No sexual predilection was found in the Queensland survey[51]. From other publications, it appears that young males are more at risk than females[53,73,75,91]. In horses, geldings seem to be more at risk to sarcoid than stallions or mares[53]. In a population of donkeys (n=80) in the UK, the proportion of males and females with sarcoid was 3.8% and 2.2% respectively[74].

Multiplicity of sarcoid tumours

Sarcoids can occur on any part of the body, either singly or in clusters[23]. In the Queensland survey, 22.2% of horses affected by sarcoid had multiple lesions. Of these approximately 63% had two, 25.7% had three, 2.8% had four, 5.7% had five and 2.8% had more than five lesions respectively[51]. In a South African survey, only 3.4% of horses had more than one sarcoid[7]. The average number of lesions per horse in continental Europe is about three compared to 25-30 lesions per horse in the UK[35]. Of the 51 sarcoid cases presented at the Veterinary School of the University of Glasgow, 29% of animals had one, 47% of animals had 2-5 and 24% had more than five lesions respectively[91].

Type of sarcoid

In Australia, the fibroblastic form is the commonest type while the verrucose form is the most common type in the UK[35]. Voss found that the type of transferred sarcoid differed between different sites of the body. Lesions on the neck tended to be verrucose while lesions on the limbs tended to be more fibroblastic in appearance[95]. No significant correlation was however found between the lesion location and the type of lesion in another study[91]. In a study of 421 sarcoid lesions in horses, the fibroblastic type was the most common, followed by mixed and least frequent was the verrucose type[87].

Location on body

Sarcoids can occur on any part of the body[23]. Location of sarcoid tumours in horses varies between clinics and the different geographic locations where studies have been conducted[45]. In the Queensland survey, 40% of sarcoids occurred on the lower leg, 18% on the upper leg, 1% on the abdomen, 15% on the chest and trunk, 25% on the head and neck and 1% on the genitalia, respectively[51]. In the latter study, sarcoids were most commonly found on areas on the horse most susceptible to trauma. In another study of 421 sarcoid lesions seen at the Veterinary Pathology Institute of the Justus-Liebig University in Germany, the most common sites were the ventral body regions, head, neck and other sites of thin skin[87].

Knottenbelt noted that the commonest sites were those areas with thin skin, limited hair cover and with a tendency to sweat. In the UK, sarcoids were rare on the upper trunk, back and neck. When they did occur at these sites, it was usually associated with traumatic damage to the skin. Lesions on the distal limb were also rare and usually observed following skin damage[35].

In the South African survey, (n=143), 46.5% of sarcoids occurred on the head, 19% on the limbs, 17.2% in the inguinal area, 8.6% on the chest, 6.8% on the abdomen, and 1.7% on the neck, respectively. Of the sarcoids located on the head, 41% were situated on the ears, 15% on the eyelids and 44% on the rest of the head. Of the abdominal sarcoids, 71.4% were located in the inguinal area[7].

Predilection sites summarized from five studies in various countries and representing 662 sarcoids were: 45.8% on the limbs, 31.6% on the head and neck, 8.8% chest and trunk, 6% abdomen and flanks, 3.6% preputial, 0.3% at castration wounds and 3.6% at other sites, respectively. Sites predisposed to trauma or contact with other sarcoids also show a higher tendency for sarcoid development[84].

In a population of donkeys in the UK, 87% of lesions in males occurred on the head (40%) and paragenital (47%) areas. In females the lesions were more evenly distributed, but with significantly more lesions on the ventral abdomen (27% compared to 4.2% in males). The combined frequency of sites was as follows: head 37.5%, ventral abdomen 13.5%, paragenital 35%, Limb 10% and other sites 4%, respectively[75].

In the study of sarcoid cases referred to the Veterinary School of the University of Glasgow (n=51), the para-genital (50.2%) and ventral abdominal (26%) areas were found to be the sites most commonly affected. The head and limbs accounted for 14.2% and 9.6% of sarcoids, respectively[91].

Various authors state that predilection sites for sarcoid development are the areas on the body that are most frequently subjected to trauma[30,70,94,95]. These findings are supported by the results of a study which found that latent papillomavirus is activated at sites of chronic irritation and hyperplasia in the epidermis[81].

Prevalence and Incidence

Increased incidence of sarcoid was evident over a 10-year observation period in Queensland[51]. Prevalence of sarcoid in horses presented to large veterinary hospitals ranged from 0.5 to 2%. Up to 90% of skin tumours in horses were equine sarcoid. Sarcoid can account for 0.7-2% of all equine clinical cases presented to veterinary hospitals[45].

In South Africa, from 1935 to 1974, equine sarcoid accounted for 38% of all skin tumours from horses submitted to the Pathology Section of the Onderstepoort Veterinary Institute for histopathology[7]. This agrees with an earlier survey which reported that sarcoid accounted for 37% of all skin tumours in domestic horses in South Africa[30].

The overall prevalence of equine sarcoid in continental Europe is 2.5-3% compared to 6-8% in the UK[35]. Reid *et al*., documented a prevalence of 2.9% in a population of donkeys in the UK. The incidence in the donkey population peaked at 1%[75]. Based on the cases at the Washington State University (Pacific North-West of the USA), Ragland *et al*., reported a sarcoid incidence of 0.0049 per hundred per week for horses. For an epizootic in a population of horses in eastern Washington, the same authors reported an incidence of 1.8 per 100 horses per week for a 6-week period[69].

2.3.10 PATHOLOGY OF SARCOIDS

Equine sarcoid has also been described as a fibrosarcoma[51]. Sarcoids characteristically show linear or focal dermal thickening of pale colour and firm texture and the epidermis varies from thick, rough and hyperkeratotic in texture to ulcerated. They can also appear as movable sub-cutaneous masses with overlying intact skin. Although sarcoids do not fulfil all the criteria to be considered malignant (they are non-metastatic), they are locally invasive and recurrent[45]. Horses with sarcoid are often reported to have reduced performance. The systemic effects are not fully understood but in ulcerated, haemorrhaging lesions, loss of red blood cells and protein can be significant[35].

Differential diagnoses for sarcoids

Although relatively characteristic in gross appearance, sarcoids often need to be distinguished histologically from exuberant granulation tissue ("proudflesh"), keloids, equine papillomas, rare mesenchymal tumours (fibroma, fibrosarcoma, neurofibrosarcoma or neurofibroma), habronematosis and other infectious and non-infectious granulomas[45,84], equine eosinofilic granulomas, melanomas, lymphosarcoma, linear hyperkeratosis, dermatophytosis, blisters, burn and rub marks[23]. Pox lesions in the form of Uasin-Gishu disease may also be confused with equine sarcoid (J Williams, Department of Para-clinical Studies – Pathology Section, Faculty of Veterinary Science, University of Pretoria, pers. comm., 2007)

Granulation tissue has no epidermal component and is more organized with a parallel vascular component. Keloids are fibrous masses composed mainly of collagen with few cells. Equine papillomas are histologically more epidermal, multiple, cauliflower-like and often regress

spontaneously. Neurofibromas are always associated with nerves, show malignant changes and are usually found in the brachial plexus area[84]. Because of difficulties with histological diagnosis, identification of BPV-like DNA may become the new standard for diagnosis of equine sarcoid[66].

Macroscopic classification

Classification of sarcoid tumours has changed over the years. In the 1970's, sarcoids were classified according to morphologic variations as verrucose (wart-like), fibroblastic (resembling granulation tissue) and mixed. Any of these can be either sessile or pedunculated[70]. Later, the classification was broadened to include verrucose-, fibroblastic-, mixed verrucose, fibroblastic and flat sarcoid on gross appearance[45]. Pascoe and Knottenbelt subsequently described five distinct clinical sarcoid types namely occult, verrucose, fibroblastic, nodular and mixed sarcoids[63]. The latest classification uses six classes namely occult, verrucose, nodular, fibroblastic, mixed and malevolent sarcoid for classification on gross clinical appearance[35]. This classification is described below.

i. Occult sarcoid

Lesions manifest as a slightly thickened area of skin with mildly roughened surfaces devoid of hair. This type seems to favour the skin around the mouth, eyes, neck and hairless areas at the medial aspect of the forearm and the thigh. Occult sarcoid is slow growing and can progress to the verrucose type. Trauma to an occult sarcoid can also lead to transformation to the fibroblastic type. They usually appear as grey hairless, often circular lesions and often with changes in hair coat colour, thickness and density[23,35].

ii. Verrucose sarcoid

Verrucose sarcoids are grey, scabby or warty growths and may sometimes have small, shot-like solid nodules within them. They may ulcerate and can either be small well-defined lesions or cover large ill-defined areas[35]. If they progress to the fibroblastic type following surgical intervention or trauma, they often present as a mixed sarcoid during the transformation while still having characteristics of both verrucose- and fibroblastic types[84]. Verrucose sarcoids show a predilection for the face, body, groin and sheath areas. They are usually slow growing with a horny, cauliflower like appearance[35]. They can become aggressive and transform to fibroblastic type on injury[23].

iii. Nodular sarcoid

These lesions are usually sub-cutaneous movable nodules with a predilection for the groin, sheath, thigh, axilla and eyelids. There may be dermal attachment to the underlying tissues, which will restrict sub-cutaneous movement depending on the site and the type of underlying tissue. With progression, the overlying skin may ulcerate and the lesion then transforms to the fibroblastic type[23,35]. According to Knottenbelt, those without skin involvement are called Type A and those that are firmly attached to overlying skin are called Type B. They can vary in size from a few millimetres to 30 cm in diameter, occur singly or in multiples and may also occur within an occult or verrucose lesion[35].

iv. Fibroblastic sarcoid

This type of sarcoid is usually found on the groin, eyelid, lower limbs, and previous wound sites. It sometimes develops when other types of sarcoid are subjected to trauma. Fibroblastic sarcoids have a variable appearance. Some are well-circumscribed fleshy fibrous nodules covered by intact epidermis while others are large ulcerated masses, which easily haemorrhage and are often covered by necropurulent debris. This type is usually aggressive and will spread locally into the dermis[23,35]. Knottenbelt classified them as Type I when they occur on a pedicle and as Type II when they have a wide base[35].

v. Mixed sarcoid

This is a mixture of two or more types that can develop at any site (most commonly on the head, axilla and groin). Most lesions eventually progress to this type[35].

vi. Malevolent sarcoid

This is the most aggressive type in which the tumour spreads through the skin with cords of tumour tissue interspersed with nodules and ulcerating fibroblastic lesions. There are usually some overlying verrucose and occult lesions. This type is rare but is mostly encountered in the face, medial thigh and elbow regions[35].

Histopathology

A histopathological diagnosis is based on the presence of a capillary-poor fibroblast proliferation of moderate to high cell density. Individual cells are fusiform or spindle-shaped, forming whorls, interlacing bundles and haphazard arrays with one another. They vary from

slender cells with elongated nuclei to plump cells with large, irregular, more pleomorphic nuclei. Cytoplasmic boundaries are ill defined. The mitotic rate is invariably low and the amount of collagen produced varies considerably. Fibroblasts orientated perpendicular to the basement membrane of the epidermis (picket fence pattern) occur in a high percentage of equine sarcoids. Hyperkeratosis, hyperplasia and acanthosis is usually seen if the epidermis is intact except in flat sarcoids, where the epidermis can be atrophic with marked hyperkeratosis. Slender to branching elongated epidermal rete ridges/pegs extending into the sarcoid directly below the epidermis are characteristic of most sarcoids where the epidermis is still intact[45]. Epidermal inclusion cysts (resembling dilated degenerated hair follicles) containing keratin may be present[23]. Pseudoepitheliomatous hyperplasia, ulceration and inflammation of the epidermis were seen in up to 50% of equine sarcoids[98].

Ultra-structurally, sarcoid matrix consists of bundles of collagen fibres laid down in whorls and undulations around fibroblasts. The endoplasmic reticulum in these fibroblasts is well developed indicating a high level of synthetic activity. Fine fibrillar material between collagen fibres is thought to be mucopolysaccharide. Although the organization of Type III collagen within sarcoid matrix is different from that of normal skin, the gene expression in the sarcoid cell is similar to that in normal dermal fibroblasts. The increased levels of collagen synthesis by the sarcoid cell may be due to the effect of unintegrated viral DNA on cellular activity[98]. A non-transforming derivative of the myeloblastosis-associated virus (MAV-20) has been found to result in a 3-fold increase in collagen production in chicken embryo fibroblasts[6].

The exact histopathological appearance of equine sarcoid is however still subject to debate[7]. The initial description of the lesion in horses had similarities to sarcoid in humans, so the term equine sarcoid was used[30]. Both lesions are biphasic in nature, being composed of both dermal and epidermal elements[7]. The epidermal component is not necessarily always present. Some authors also refer to sarcoids as fibropapillomas[70]. Baker and Leyland on the other hand diagnosed 35% of 124 equine skin tumours as fibromas and only 12.9% as sarcoid[5]. They mentioned the difficulty of differentiating between fibroma and equine sarcoid on histological basis. Because of the absence of an overlying epithelial component and the absence of “picket fence pattern” and rete pegs, many lesions were classified as fibromas. According to them, fibromas have fibres of variable maturity with random or irregular arrangement whilst in sarcoid, fibres are of uniform development and mostly run in two directions.

The dermal component consists mostly of immature fibroblasts lacking distinctive histological pattern[70]. Jackson described fibroblasts as running in all directions with varying degrees of maturity, tending to resemble those found either in fibrosarcomas (when immature) or fibromas (when mature). According to him, the epithelial component of a sarcoid is initially prominent, but as the dermal component expands, the epithelial part is displaced, ulcerating initially and later disappearing. In later stages therefore, the biphasic nature of sarcoids is no longer present[30].

2.3.11 TREATMENT

The numerous different treatment options described in the literature are a good indicator that there is no one universal practical and successful treatment regimen available[20,23,45,49]. Sarcoid cases vary in their response to treatment and the prognosis depends on the site, the type of sarcoid and the method of treatment[35,45]. The following are the most common treatment methods that have been used:

Surgical excision

Between 50 and 64% of sarcoids treated by conventional surgical excision recurred within three years[45]. Tumour re-growth was a common occurrence regardless of the method of treatment used[23]. Recurrence rates of between 50 and 70% have been reported elsewhere following surgical removal. Type A nodules were sometimes good candidates for surgical removal. Lesions around the eye should not be removed surgically without careful consideration[35]. With surgery, a margin of 1.5 – 2.5 cm around the lesion is suggested to prevent leaving affected tissue behind. Recurrence or hyperproliferation after surgical excision could be due to activation of latent BPV in normal tissue surrounding sarcoids[20].

Cryosurgery

Cryosurgery resulted in regression in 60 to 100% of cases in different reports[45]. Cryosurgery also resulted in spontaneous regression of untreated sarcoids in horses with multiple tumours, suggesting a cryo-immune response to sarcoid cell components[38]. Knottenbelt, however, reported a high recurrence rate after cryosurgery. It can only be used on small lesions of limited depth. It is not a practical method where an animal has many small lesions, as it is

tedious and time-consuming. Cryotherapy needs to be aggressive, and can therefore result in extensive damage to surrounding tissues. Three freeze-thaw cycles with a tissue temperature of -20ºC to -30ºC should be applied to all lesions. Thermocouples should be inserted into adjacent tissue to monitor the temperature. Surgical debulking can be used to reduce the mass of large lesions[35].

Carbon dioxide laser surgery

Carbon dioxide laser surgery may be the most reliable method of treating equine sarcoid (approximately 80% success), but the equipment needed is expensive and the use thereof requires specialist training[45].

Immunotherapy

Immunotherapy is often the preferred method of tumour treatment, as the use of toxic chemicals, irradiation and surgery can be avoided. For immunotherapy to be effective, three criteria have to be met: Firstly, tumour cells must express some antigen distinguishing them from their normal counterparts. Secondly, the antigens should be recognized as an auto-antigen to induce an immune response. Thirdly, the effector cells or anti-bodies must be able to destroy the abnormal cells[40]. Treatment using BCG is the most commonly applied immunotherapy technique. BCG is a non-specific immunomodulator which has to be injected in close association with tumour cells (intralesional). Patients should be immuno-competent with a limited sarcoid burden[45]. Only sarcoid cells undergo necrosis after BCG treatment and not normal cells[94]. This suggests that BCG may induce sarcoid specific immunity. Immunotherapy using BCG is however only universally successful in small periocular sarcoids and largely ineffective in sarcoids located at other sites[45]. BCG may cause anaphylactic shock, especially after repeated injections, and premedication with flunixin meglumine and corticosteroids has been suggested to avoid this[94]. The use of BCG in lesions located on the limbs may be contra-indicated as these lesions often become worse after treatment. BCG powder is dissolved into 0.5 – 1.5ml (depending on lesion size) of the diluent provided and injected intra-lesionally using a thin needle of appropriate length. Five to nine weekly injections may be necessary[35].

Good results have also been reported with once weekly intralesional or intravenous injections of Eqstim (*Propionibacterium acnes*, Neogen Corporation, Lansing MI) injection for 4-6 weeks[23].

A decrease in recurrence rates after surgical excision in primary and recurrent sarcoids was reported using auto-vaccination in horses in combination with surgery[55]. On the other hand, Knottenbelt mentioned that the use of autogenous vaccination is contra-indicated for all types of sarcoid[35].

Interstitial brachytherapy

Various isotopes have been used for interstitial brachytherapy with responses varying between 50 and 100% success. The cost may be significant and horses must be kept in a radiation safety approved area. It may however be of use in aggressive, recurrent and surgically inaccessible sarcoids[45]. Permanently implanted seeds of radon-222, gold-198 and removable seeds of radium-226, cobalt-60 or irridium-192 have been used[23]. Radiation has been found to give the best results for sarcoid treatment, but cost and availability limits the use to selected cases in valuable animals[35].

Radio-frequency current-induced hyperthermia

Radio-frequency current-induced hyperthermia has been successful in some cases but needs further research[45].

Chemotherapy

a) Topical chemotherapy

Topical chemotherapy using xanthates or 5-fluorouracil has been used successfully for smaller sarcoids. Implants containing cisplatin or 5-fluorouracil have showed promising results but further investigations are needed[45]. Daily topical application of the antimetabolite 5-fluorouracil and the irritant cathartic podophyllin has been used with some success in sarcoid cases[23].

A bloodroot extract that contains *Sanguinaria canadensis*, puccoon, gromwell, distilled water and trace minerals (AnimexTM, NIES, Inc., Las Vegas, NV) is an escharotic ointment that penetrates the lesion, killing affected cells while leaving surrounding normal tissue intact. Some consider this as the treatment of choice for small sarcoids that can easily be bandaged[23]. It results in a rapid response within 7 to 10 days. A similar topical treatment containing a caustic agent and bloodroot extract (XXTERRATM, Larson Laboratories, Inc., Fort Collins,

CO) is believed to change the antigenicity of affected cells so that the immune system recognizes them as being foreign, resulting in tissue rejection[23].

Another topical treatment (AW-3-LUDES, D.C. Knottenbelt, Division of equine studies, Leahurst, Neston, South Wirral, UK) that has been used with success contains heavy metals, and the antimitotic compounds 5-fluorouracil and thiouracil. Successive or alternate-day treatments for 3 to 5 days results in a response of preferential necrosis and sloughing of sarcoid tissue within 5 to 10 weeks in about 70% of cases[23,35].

b) Intralesional chemotherapy

Intralesional injection of cisplatin in oil has provided extremely promising results[90]. This method is especially effective in small nodular and fibroblastic lesions. A dosage of 1mg Cisplatin per gram of tumour tissue is suggested, but oil emulsions contain a low concentration (0.5mg/ml) making it difficult to inject the desired volume into a lesion[35].

Homeopathy

Homeopathic remedies have produced variable and unpredictable results in the treatment of sarcoids[35].

Chapter 3

Article — Artikel

Descriptive study of an outbreak of equine sarcoid in a population of Cape mountain zebra (*Equus zebra zebra*) in the Gariep Nature Reserve

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ABSTRACT

An outbreak of equine sarcoid occurred in a population of Cape mountain zebra *(Equus zebra zebra*) at the Gariep Nature Reserve located in the southern Free State Province of South Africa in 1996. The course of the outbreak during 1996 to 2003 is described. During this period the average population size was 69 animals. Initially (1996) all affected animals were removed from the population. New cases continued to manifest and the incidence varied between 4.6 % and 17.6 %. Prevalence reached 24.7 % in 2002. No sexual predilection was noticed in the 39 recorded cases. Of the affected individuals, 64 % had a single lesion and no animal had more than 4 lesions. In males, the majority of lesions occurred in the inguinal area (55.17 %), whereas in females they mostly occurred on the head and neck (41.38 %). Lesions can increase 260 % in size annually and may impede movement.

Key words: Cape mountain zebra, equine sarcoid, Gariep Nature Reserve, tumour.

Nel P J, Bertschinger H, Williams J, Guthrie A J, Thompson P **Descriptive study of an outbreak of equine sarcoid in a population of Cape mountain zebra (*Equus zebra zebra)* in the Gariep Nature Reserve**. *Journal of the South African Veterinary Association* (2006) 77(0): 00–00 (En.). Free State Department of Tourism, Environmental and Economic Affairs, Private Bag X20801, Bloemfontein, 9300 South Africa.

INTRODUCTION

Sarcoids were first described and characterised as a clinical entity in the equine species in South Africa by Jackson in 1936 Equine sarcoid was found in different studies to be the most common skin neoplasm in horses, mules and donkeys[3,7,12] The World Health Organizsation (WHO) Tumour Classification System lists the sarcoid as a benign tumour and although it can be locally invasive, it is strictly limited to the skin and directly-underlying tissues and does not spread to internal organs[9]. There is considerable evidence that equine sarcoid is caused by a virus closely related to or identical to bovine papilloma virus (BPV)[12]. Intra-dermal inoculation of horses with cell-free extracts from bovine skin tumours containing BPV has been shown to cause lesions resembling equine sarcoids[12]. In a study of histologically confirmed sarcoids from 58 horses, DNA for BPV-1 and BPV-2[18] could be amplified from every sample.

The Cape mountain zebra (CMZ, *Equus zebra zebra*) is described as a subspecies of *Equus zebra*; the mountain zebra[14]. The species is vulnerable[6] and occurs as a number of isolated populations, 1 of which is in the Gariep Nature Reserve (GNR).

Sarcoids were first observed in the CMZ of GNR in June 1995 when nature conservators reported 'large growths' on some animals. In January 1996, tumours from 2 animals were sampled and a histological diagnosis of equine sarcoid was confirmed (Pathology Section of Onderstepoort Veterinary Institute). Four similar diagnoses in the GNR were made later in 1996.

The aim of this study was to document the outbreak of equine sarcoid in the GNR for the period 1995 to 2003. During this period the CMZ population varied between 50 and 75 animals (average population size was 69) and 39 cases of CMZ with sarcoid were observed.

MATERIALS AND METHODS

Main study area – Gariep Nature Reserve

The GNR is situated in the southern part of the Free State Province of South Africa along the northern shore of the Gariep Dam. The GNR (6000 ha) consists of steep flat-topped hills and undulating plains. The geological formations consist mainly of the Beaufort group sandstone and shale, which is shaped by dolerite dykes and sills. Average annual rainfall is 350 mm (range 150–500 mm) and the vegetation is classified as eastern mixed Nama Karoo[10]. Temperatures vary from a minimum of –10 °C in winter to a maximum of 42 °C in summer. The northern side of the GNR, which is game-fenced, borders commercial farmland with sheep, goats, cattle and horses.

Animals and population dynamics

According to the ecological management plan of the GNR, the CMZ population is managed to keep the population at between 50 and 70 animals. Excess animals are removed by live capture when the number approaches 70 animals. In 2002, a detailed investigation was conducted on the population. The 70 animals in the population were grouped into 16 herds, of which 11 were breeding herds with an average of 5 zebras and constituting 71 % of the population total. The rest consisted of bachelor groups with an average of 4 zebras per group, making up the remaining 29 % of the population. The male:female ratio was 1.2:1 and 70 % of the animals were 3 years and older. No reliable data are available on contact rates between herds. Young males and females leave the breeding herd when they reach sexual maturity. Males join bachelor herds. Females are either gathered by a male from a bachelor herd to form a new breeding herd or they join other newly formed breeding herds. Herds sometimes mix if forced by some source of perceived danger (like chasing them for capture purposes) when they use the same escape route. Once left undisturbed they separate again.

Sarcoid-affected CMZ that had survived up to the end of 2003 were moved to a separately fenced area in the GNR in order to decrease to contact rate between affected and non-affected animals.

Hands-off field observations

The CMZ population at GNR was examined annually using standard ×10 field binoculars. Where it was not possible to

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Received: August 2006. Accepted: November 2006.

get close to the animals, a spotting scope (×20 magnification) was used. An attempt was made to examine both sides of each animal in every herd. During these visual observations, the following were recorded on affected zebras: presence, number, estimated size (diameter) and location of tumour-like skin lesions as well as age and sex and the total number of individuals in the herd.

From field observations, the following epidemiological parameters were calculated:

- Number of affected animals in the population (prevalence). Animals which recovered after treatment trials were regarded as positive for the sake of prevalence calculations.
- Population at risk, which includes all animals in the population visually free of sarcoids.
- Adjusted population size (actual population number –50 % of live removals of healthy animals + 50 % of births – 50 % of natural mortalities not related to sarcoids) for a 12-month period
- Number of new sarcoid cases in adjusted population at risk for the previous 12-month period (adjusted incidence)
- Age classes of affected animals
- Sexes of affected animals
- Herd structure of the population (2002 only)
- Age class composition of the population (2002 only)
- Male:female ratio in the population (2002 only)

Hands-on examination

Immobilisation for examination. Excluding 1999, all affected animals found during the annual capture period were immobilised using a Paxarms (Paxarms SA) remote injection rifle and from a helicopter. The herds were screened from the helicopter in a systematic manner. While flying at a low level every animal in each herd was observed visually from the sides and back for any cutaneous growths. Every effort was made to capture all affected animals (including recovered) in each herd for evaluation. Only 1 animal was immobilised at a time. After evaluation on the ground the animal was revived before continuing with the same herd.

Chemical immobilisation was performed using 24–26 μg/kg etorphine hydrochloride (M99, 9.8 mg/mℓ, Novartis AH) in combination with either 0.5 mg/kg azaperone (Azaperone, 100 mg/mℓ, Kyron Laboratories) or 31–43 μg/kg detomidine hydrochloride (Domosedan, 10 mg/mℓ, Novartis AH). The etorphine HCl was reversed with an intravenous bolus of approximately 50 μg/kg diprenorphine hydrochloride (M5050, 12 mg/mℓ, Novartis AH).

Observations. All affected animals were identified using individual ear notch combinations, plastic ear tags of different colours and shapes and photographs of each side using a Sony Cybershot 2.3 Megapixel digital camera. During hands-on examination, the following data were recorded: date of immobilisation; age[19]; sex; ear notch number; ear tag colour, sarcoid location and dimensions. The location of each lesion was noted as 1-location occurrence. Any treatments or other relevant information were also recorded. With the notch numbering system, animals were numbered sequentially from 1 onwards. A biopsy of each lesion was collected in 10 % buffered formalin for histopathological confirmation of the diagnosis (results not presented here).

From the hands-on examinations and the field observation data, the following parameters/tendencies/features were noted:

- Predilection sites of the tumours on the body
- Differences in predilection sites between male and female animals.
- Tumour multiplicity and the difference between male and female animals.

Age at first onset (age determination was done using dental wear patterns[19] The initial animals examined in 1996 were only classified into broader age groups and some cases were only visual observations from a distance, without exact ages being established.)

- Change in sarcoid size

Sarcoid tumour size

Owing to the fact that many sarcoids had irregular shapes, the length, width and height were measured and used to calculate a volume index (V_i).

The formula for the volume of a sphere was the used to calculate V_i using the average of length, width and height as the diameter. The formula used for the volume of a sphere is: $V = (\pi/6)d^3$ where V = volume and d = diameter.

Comparative investigations

For the sake of comparison, the prevalence of sarcoid-resembling tumours was investigated in horses and Burchell's zebra *(Equus quagga burchellii*) in the Free State and CMZ in the Mountain Zebra National Park (MZNP) on a once-off basis during 2004. The Burchell's zebra *(Equus quagga burchellii)* were observed in 4 Free State provincial nature reserves, namely Tussen die Riviere, Maria Moroka, Koppies Dam and Sandveld. Burchell's zebra were examined using the same method as described for the CMZ at GNR (see 'Hands-off field observations' above). 'Tussen die Riviere' Nature Reserve (23 000 ha) borders the GNR on the eastern side and has the same vegetation type[10]. Maria Moroka Nature Reserve (5500 ha) in the central Free State and Koppies Dam Nature Reserve (area 2457 ha) in the north both fall within the moist cool highveld grassland vegetation type[10]. Sandveld Nature Reserve (14 000 ha), on the northwestern border of the Free State, consists of Kimberley thorny bushveld vegetation type[10]. The domestic horses investigated were on 2 commercial farms, both American saddle horse studs that border the GNR on the north. Cattle are also present on these farms and farmers were questioned about the presence of skin tumours on horses and warts in cattle. The CMZ (n = 300) in the MZNP in the Eastern Cape Province were also examined in 2004 for macroscopically visible sarcoid-resembling lesions. This population is possibly more out-bred than the GNR population. The MZNP (28 000 ha) has eastern mixed Nama Karoo (northern section) and southeastern mountain grassland (southern section) vegetation types[10].

RESULTS

Prevalence and incidence

Basic data on the dynamics of the CMZ population at Gariep are presented in Table 1. Because the population seldom remained static over a period of a year, the adjusted population size was calculated and used to determine the adjusted population at risk and adjusted incidence as well as the prevalence as presented in Table 2.

Monitoring of CMZ in GNR is not done at the intensity where all mortalities and births are recorded. The ruggedness of the terrain also makes the task very difficult. For this reason the net change was calculated from year to year based the difference between births and natural mortalities.

The sarcoid prevalence in 4 populations of Burchell's zebra (n = 427) on other provincial nature reserves in the Free State was nil. With the exception of a prevalence of 0.9 % in the CMZ at MZNP (n = 219), no sarcoid tumours were seen in either the Burchell's zebra populations, or the 2 horse populations (n = 80) adjoining the GNR.

Age and sex distribution

Distribution of sarcoid lesions according to age group and sex, based on first observation at GNR is presented in Table 3. Eleven (3 males and 8 females) of the 39

Table 1: **Basic population dynamics data for Cape mountain zebra (CMZ) from Gariep Nature Reserve, 1996–2003.**

Year	Population size	Introductions (sarcoid-free)	Sarcoid-affected CMZ removed from population*	Live removals of healthy individuals	Net change in population to following year**
1996	64	0	6	5	+ 18
1997	71	5	1	17	0
1998	58	0	2	0	+ 6
1999	62	0	0	5	+ 8
2000	65	0	2	0	+ 9
2001	72	0	2	0	+ 7
2002	77	0	7	0	+12
2003	82	0	19	20	+ 3

*This column includes all mortalities (known and assumed), euthanased animals, and live removals to quarantine areas.
**Births minus natural mortalities during the year.

Table 2: **Some population dynamics and epidemiological data for Cape mountain zebra from Gariep Nature Reserve, 1996–2003.**

Year	Population size	Adjusted population size	Adjusted population at risk	Number of new cases	Adjusted incidence (%)	Prevalence at end of period (%)
1995–1996	64	70.5	64.5	6*		9.4
1996–1997	71	65.0	62.0	3	4.65	4.20
1997–1998	58	61.0	59.0	0	0.00	3.40
1998–1999	62	63.5	60.5	3	5.08	4.80
1999–2000	65	69.5	62.5	4	6.61	10.80
2000–2001	72	75.5	59.5	11	17.60	22.20
2001–2002	77	83.0	64.0	5	8.40	24.70
2002–2003	82	74.0	54.0	8	12.50	24.40

*This number of cases could possibly have originated during a period of more than 12 months.

cases were not included in Table 3 on account of inaccurate age assessment (hands-off observations). In young CMZ, the first sarcoids were observed at 3 years of age. The difference in case numbers between age groups is, however, not statistically significant ($\chi^2 = 10.2$, $P < 0.61$).

Of the 39 sarcoid cases recorded from 1996 to 2003, 19 (48.7 %) were males and 20 (51.3 %) females. The difference in sex ratio of sarcoid-affected animals at different ages was found to be not statistically significant ($\chi^2 = 12$, $P < 0.44$. In 2002 the CMZ population ($n = 82$) consisted of 55.5 % males ($n = 40$) and 44.5 % females ($n = 32$). The remaining 5 foals and 5 subadults were not sexed. During the next year, 8 new cases were recorded, 3 of which were males and 5 females. Thus 7.5 % of the males counted in 2002 developed sarcoid tumours, compared with 15.6 % of females.

Predilection sites and multiplicity

Table 4 shows the anatomical distribution of all the sarcoids recorded during the study period. Several animals had more than 1 lesion in different locations. A total of 58 lesions were recorded on 39 CMZ; 29 on males and 29 on females.

Data on the number of tumours per animal are given in Table 5. The majority of cases ($n = 25$) had only 1 lesion. No animal had more than 4 separate lesions on the body.

Rate of sarcoid growth

From 2001 to 2003, the average volume index of lesions on 1st observation ($n = 20$) was 76 cm^3. The epidemiological study coincided with a treatment trial where most of the infected animals ($n = 22$) were treated by 1 of 3 (autogenous vaccination, BCG treatment and surgery or a combination thereof) methods which resulted in a combined tumour regression rate of 77.5 %. The non-responders had an average annual increase in tumour size of 260 % ($n = 5$).

Table 3: **Distribution of sarcoid cases ($n = 29$) according to age groups and sex in the Gariep Cape mountain zebra population, 1996–2003.**

Sex	<3 years	3–6 years	7–10 years	>10 years	Total
Male	0	6	5	5	16
Female	0	5	4	3	12
Total	0	11	9	8	28

DISCUSSION

The outbreak of a disease in a threatened wildlife population is always a cause for concern. The approach to a disease outbreak in wildlife differs markedly from the approach in domestic animals. In wildlife, the objective is to correct the natural balance between the animal, the ecosystem and the disease agent to ensure the long-term survival of the population as opposed to the salvage of the individual animal for companionship, performance of reproductive purposes in domestic animals.

The outbreak was studied in order to determine the course of the disease in the population and the effect it would have on the dynamics of the populations. Other aspects of the sarcoid outbreak were studied parallel to the epidemiology but falls outside the scope of this article.

Initial PCR and restriction enzyme digestion studies done on lesions from the Gariep CMZ resulted in BPV detection in all the samples[28]. Both BPV I and BPV II were found in the samples tested. This confirmed the assumption that the aetiology is similar to that in domestic equids.

Table 4: **Location of sarcoids in male and female Cape mountain zebra, 1996–2003.**

Location	Male lesions (*n* = 29)	Male distrib %	Female lesions (*n* = 29)	Female distrib %	M + F total	M + F %
Head and neck						
Ear	1	3.45	4	13.79	5	8.62
Poll	1	3.45	0	0.00	1	1.72
Cheek	0	0.00	1	3.45	1	1.72
Lower jaw	2	6.90	3	10.34	5	8.62
Throat	1	3.45	1	3.45	2	3.45
Eye	0	0.00	3	10.34	3	5.17
Neck	1	3.45	0	0.00	1	1.72
Head and neck combined	6	20.69	12	41.38	18	31.03
Trunk and legs						
Chest and trunk	3	10.34	4	13.79	7	12.07
Inguinal	16	55.17	8	27.59	24	41.38
Perineal	0	0.00	1	3.45	1	1.72
Distal limb	0	0.00	1	3.45	1	1.72
Elbow medial	0	0.00	2	6.90	2	3.45
Knee medial	4	13.79	1	3.45	5	8.62
Trunk and legs combined	23	79.31	17	58.62	40	68.97
Total	29	1.00	29	1.00	58	2.00

BPV-DNA found in normal skin of horses affected with equine sarcoid[4,11,27] could indicate viral latency. BPV was not found in the normal skin of horses without equine sarcoid[4,18,26]. Latency could make the determination of incubation period difficult. Activation of latent papillomavirus genomes by chronic epidermal irritation has been documented in *Mastomys natalensis*[24]. It has also been noted that sarcoids in horses tend to occur in areas of skin damage[9]. It is possible that for BPV DNA to be activated, some form of epidermal damage or irritation is necessary for the activation of transacting factors which binds to the regulatory regions causing expression of the viral genome which then leads to sarcoid development.

Incubation period was not determined in CMZ. In a case of experimental autotransmission between horses by topically inoculating scarified skin, the incubation period was 6 months while another study indicated that *incubation period* differed between homologous transmission (115 days) and autologous transmission (57 days) in transmission studies conducted[16,29]. Incubation periods of 5 and 10 months have been noted in 2 donkeys inoculated intradermally with a cell-free sarcoid extract from a horse[18]. It is extremely difficult to determine incubation period in an extensive CMZ population, as lesions are only seen when they reach a certain size.

Transmission studies in horses have been relatively unsuccessful. Scarified skin seems to be more receptive to sarcoid development that sub-cutaneous or intradermal inoculations[12,17,29]. Although there are strong indications that flies are responsible for the spread of sarcoids from one site to another in an individual horse, it is uncertain whether insects are responsible for transmission between horses. Close association between animals was shown to be a risk factor in transmission of BPV (Reid *et al.* 1994)[22]. As no viral genomes have been found in the blood of affected horses, spread *via* the bloodstream is not regarded as a method of spread[9]. No BPV-DNA was found in blood or normal skin of sarcoid-affected CMZ from GNR in a preliminary study (E van Dyk, Faculty of Veterinary Science, University of Pretoria, pers. comm., 2004).

The *locations* of sarcoid lesions in the Gariep CMZ population were predominantly in areas of thin skin and a sparse hair cover (Table 4) and corresponds with observations in horses[9]. This may support the theory of an insect vector[9], as these sites could be the preferred sites for a biting insect vector. It is, however, a hypothesis that will require further investigation. It is interesting to note that no sarcoid lesions were ever recorded in the 2 horse studs adjoining the GNR. Although the CMZ do not have direct contact with the horses or cattle on the adjoining farm land, they do sometimes graze within a few hundred metres from them. One of the farmers adjoining the GNR has seen isolated cases of warts on his cattle. It is possible that this could have been the source of the papilloma virus for the initial infection of the CMZ. The fact that no horses developed sarcoid could be an indication that the Gariep CMZ are more susceptible to the development of equine sarcoid compared with the domestic horses occurring next to the reserve. Although the reason for this increased susceptibility has not been proven, speculation and initial genetic results suggest that inbreeding depression may be one of the factors responsible[23].

When the Gariep CMZ population was examined in 1996 after the first reports of 'skin growths' in late 1995, the *prevalence* of affected animals was 9.4 %. After all the visibly affected CMZ were removed from the population in 1996, the prevalence was 4.6 % in 1997, after which it increased gradually to about 24 % in 2002/03. Owing to the rugged terrain and level of monitoring on the ground, few carcasses were

Table 5: **Number of sarcoid tumours per animal in the Gariep Cape mountain zebra** (*n* = 39).

Number of tumours	Male	Female	Total	Total %
1	10	15	**25**	64.10 %
2	6	4	**10**	25.64 %
3	2	1	**3**	7.69 %
4	0	1	**1**	2.56 %
Total	18	21	39	

found. Sarcoid-affected animals not found on follow-up investigations were presumed to be dead. During 2003 all affected animals ($n = 16$) were moved to another camp in order to decrease the contact rate and possible transmission between affected and non-affected. From 1996 to 2003, a total of 86 animals were removed from the population. This included mortalities and live removals. Thirty-nine were sarcoid affected and 47 were unaffected. The annual population number varied from 64 to 82 zebras. Mortality due to other causes is not included in these figures. The average annual recruitment was 17.8 % for the study period. Should all sarcoid-affected individuals were removed as soon as they were seen, the average annual productivity of the population would have only been 9.8 %. If natural mortality is deducted, the population comes very close to being stagnant. Although no sarcoid cases were found during the once-off investigation at Maria Moroka NR, it is known that from 1992 to 2004, 1 case of equine sarcoid in a Burchell's zebra was diagnosed in this reserve. The 24 % sarcoid prevalence in the Gariep CMZ population is therefore highly significant. The same would apply for the high prevalence of equine sarcoid (close to 50 %) reported for the CMZ population from the Bontebok National Park (D Zimmerman, South African National Parks Board, pers. comm., 2003).

After the initial removal of all visibly affected zebra in 1996, incidence increased from 4.6 % in 1997 to 17.6 % in 2001, after which it decreased again to 8.4 % and 12.5 % in 2002 and 2003, respectively. As prevalence increases, the contact rate between susceptible and affected zebra would increase, thereby explaining the annual increase in incidence up to 2001. The sudden drop in incidence after 2001 could have been as a result of a decrease in the number of susceptible individuals in the population but could also be as a result of other factors. Most of the susceptible animals could have been infected in previous years while susceptible offspring provided the majority of susceptible individuals.

From studies in horses it can be concluded that not all individuals are susceptible to the development of sarcoid. Until more is known about the genetics of the disease, it is not possible to predict whether an individual is susceptible to sarcoid development[9]. Initial examination of the heterozygosity status of the Gariep CMZ population using genetic microsatellite studies ($n = 5$) in 2005 indicated a significant level of inbreeding (E H Harley, University of Cape Town; Medical School, pers. comm., 1995). During 2003, 17 sarcoid-affected CMZ from Gariep were sampled in order to conduct further comparative microsatellite investigations. This was compared with CMZ populations at the Karoo National Park (KNP), Karoo Nature Reserve (KNR), Bontebok National Park (BNP) and a population of Hartmann's mountain zebra (*Equus zebra hartmannae*) from Namibia. The population from KNP is one of the 2 largest populations ($n \approx 300$) of CMZ in South Africa. The Hartmann mountain zebra (HMZ) meta-population is currently estimated at 20 000–30 000 animals[15]. KNP, KNR, GNR and BNP populations were all seeded from *the population at the Mountain Zebra National* Park. Mean heterozygosity values were lowest for the tumour-affected populations (0.386) compared with tumour-free populations (0.427) and HMZ (0.609). Mountain zebra from GNR exhibited lower heterozygosity values than BNP. CMZ from Karoo National Park (KNP) also had low levels of polymorphism and exclusion probability values. However, higher heterozygosity values were recorded when compared with BNP and GNR. Gene diversity values were lowest for tumour-affected populations (39.5 %) and highest for Hartmann's mountain zebra (52.3 %). The overall value for Nei's population differentiation parameter (*Gst'*), also an indicator of heterozygote deficit, illustrated that tumour-affected CMZ populations have a greater degree of differentiation (0.156) and deficit of heterozygote than tumour-free CMZ (0.082) and Hartmann's zebras (0.08). The mean number of alleles was lowest in the diseased populations (BNP and GNR; 2.22), with GNR having the lowest polymorphic information content. The diseased population had lower comparative allele richness values (2.33 *vs* 2.64) for the tumour-free animals and 5.896 for HMZ. The level of relatedness was high between all CMZ populations, with the diseased population showing comparatively higher values than tumour-free animals (0.29 *vs* 0.17). This investigation revealed that the 2 diseased populations have the lowest genetic variation of the 4 mountain zebra populations investigated and are representative of most extant CMZ populations. Apart from being highly inbred, these populations have a high level of genetic sub-structuring. It could thus be demonstrated that CMZ, after surviving the historically documented genetic bottleneck, have very little genetic variation left[23].

It is generally suggested by various studies that multigenerational consanguinity often significantly affects birth weight, survival, reproduction, resistance to disease, predation and environmental stress[23]. It could therefore be argue that the high sarcoid prevalence in the Gariep CMZ population could be influenced by the degree of inbreeding. The fact that 2 neighbouring domestic horse populations (average size of the 2 groups during the study period was 80 horses) did not have any sarcoid cases during the study period also indicates that the Gariep CMZ population has an abnormally high susceptibility to equine sarcoid. Out of 427 Burchell's zebra examined on other Provincial Nature Reserves in the Free State, no suspect sarcoid lesions were seen (although 1 case has been confirmed as equine sarcoid during the period 1992–2004 in Burchell's zebra from these populations). Out of 72 % of a population of CMZ ($n = 305$) which was examined visually in 2004 at the Mountain Zebra National Park, only 0.91 % of the animals had lesions resembling equine sarcoid. This could, however, be as a result of a relatively recent introduction of BPV with the potential to develop into a significant outbreak with a high incidence. The only other CMZ population previously known to be affected by equine sarcoid is the population at the Bontebok National Park (BNP) which had a prevalence of 18.75 % in 2000 and 53 % in 2002[30]. The high prevalence of equine sarcoid in inbred populations of CMZ compared with that in larger, more outbred CMZ populations, Burchell's zebra or horses is clearly significant.

No conclusive breed predilection was found amongst horses in a study in Queensland[13]. In another study it was, however, concluded that there seemed to be some breed *predilection* in horses regarding sarcoid prevalenc[12]. Quarter horses were found to have almost twice the risk of developing sarcoids compared to Thoroughbreds, while Standardbreds had a much lower risk relative to all other breed2. The association between certain equine leukocyte antigen (ELA) haplotypes and sarcoid susceptibility and expression is the strongest association between MHC genes and disease in any species. The presence of the A5 haplotype has been associated with early onset (2.9 ± 1.5 years) of equine sarcoid while the W13 haplotype has been associated with increased risk of recurrence[12,20]. The precise MHC genes responsible for sarcoid susceptibility have not yet been identified[12]. Familial tendencies towards the development of equine sarcoid have been described in 80 % of horses from a highly inbred lineage[8]. All these reports from domestic equids support the notion that the high incidence of equine sarcoid in inbred CMZ populations may be as a

result of inbreeding depression.

It has been found in horses that different **age** groups were almost equally susceptible to sarcoids, with the 1–6-year-old group the most common age at which sarcoids were diagnosed[13]. A similar trend was observed in the Gariep outbreak. The earliest age at which equine sarcoid has been found in horses is in yearlings[12]. In the Gariep outbreak, no animals younger than 3 years were seen with sarcoid. They could, however, have been infected as yearlings and with an incubation period of a few months, sarcoids would start to develop during the 2nd year and would only be large enough to be seen the following year when they were 3 years old. At the Bontebok National Park 41.6 % of the 12 sarcoid cases in CMZ, these were first seen when the animals were 2–2.5 years old compared with 10.25 % in the Gariep population that was 3 years old. The variation in the different age classes affected (Table 3) is not statistically significant ($\chi^2 = 10.2$, $P < 0.61$).

As this is a case study, the small number of samples often hampered statistical analysis.

The *sex ratio* of sarcoid-affected cases in the Gariep population is close to 1:1. Although the population was male biased during 2002 (55.5 % male: 45.5 % female) it does not necessarily mean that this was the case during the whole study period. Unfortunately the male:female ratio of the Gariep population was not determined annually. During game reduction operations, more female than male animals are usually removed. This could have resulted in the male biased population in Gariep as was observed in 2002. Of the 12 sarcoid cases from the BNP population, 33.3 % were males and 66.7 % females. This, however, is very close to the male:female ratio in BNP. No sexual predilection was found in a survey on equine sarcoid in domestic horses in Queensland1[3].

Growth rate of sarcoids have not been described in domestic horses. The only control animal (no treatments received) in the GNR study died before recapture the following year so it is impossible to determine what the natural growth rate of the tumour would have been. It is important to note that even the biopsy procedure could trigger an immune response which might influence the growth rate of the sarcoid. For determination of sarcoid growth rate, the histological diagnosis can only be confirmed once the control trial is terminated, for any surgical manipulation of the lesion may affect the growth rate by eliciting an immune response.

Although sarcoids can occur on any *part* of the body, the location of sarcoid tumours on the body of horses varies between clinics and different locations where studies have been conducted[5,12]. In a South African survey, it was found that in horses, 46.5 % of sarcoids occurred on the head, 6.8 % on the abdomen, 17.2 % in the inguinal area, 8.6 % on the chest, 19 % on the limbs and 1.7 % on the neck. Of the sarcoids located on the head, 41 % were situated on the ears, 15 % on the eyelids and 44 % on the rest of the head. Of the abdominal sarcoids 71.4 % were located in the inguinal area[3]. In a Queensland survey it was found that 40 % of sarcoids occurred on the lower leg, 18 % on the upper leg, 1 % on the abdomen, 15 % on the chest and trunk, 25 % on the head and neck and 1 % on the genitalia. It was concluded that sarcoids are commonly found on areas on the horse most susceptible to trauma[13] and areas with thin skin, limited hair cover and areas with a tendency to sweat[9]. In the UK, sarcoids are rare on the upper trunk, back and neck. When they do occur at these sites, it is usually as a result of traumatic damage to the skin. In the UK, lesions on the distal limb are also rare and usually resulted after skin damage (either new or old lesions). Predilection sites in horses summarised from 5 studies representing 662 sarcoids were: 45.8 % on the limbs, 31.6 % on the head and neck, 8.8 % chest and trunk, 6 % abdomen and flanks, 3.6 % preputial, 0.3 % at castration wounds and 3.6 % at other sites[25]. Sites predisposed to trauma or contact with other sarcoids also show a higher tendency for sarcoid occurrence. In the Gariep CMZ, 31 % of lesions occurred on the head and neck while 41.4 % of lesions in the inguinal region (Table 4). There is, however, an interesting difference between males and females. In males the inguinal lesions accounted for 55.2 % of lesions while the head and neck accounted for 20.7 % and the area medial to the stifle accounted for 13.8 %. Together these 3 locations account for 89.7 % of sarcoid lesions ($n = 29$) in males. In females, 27.6 % of lesions occur in the inguinal area, 41.4 % on the head and neck and 3.5 % on the area medial to the stifle joint. Compared with females, the male zebra has a larger surface area of thin hairless skin in the inguinal area which could make it relatively more prone to BPV transmission from biting insects in this area. Lesions on the head are also usually on areas with sparse hair cover (eyelids, ears and the soft thin-skinned area around the muzzle). In the BNP population, 33.3 % of lesions are located in the region of the axilla, 25.6 % of lesions on the trunk, usually on the ventral abdomen, and 10.3 %, respectively, on the head, chest and shoulders and the area medial to the stifle joint[30]. Although there is no scientific evidence at this stage, it is possible that different vectors with different biting site preferences could be responsible for the difference in sarcoid locations between horses and CMZ, as well as for differences between different sarcoid-affected CMZ populations. It is possible that habitat can play a role where injuries can be caused by moving through certain plants, which would attract vectors and could be an entrance point for BPV. Apart from chance (small sample size), there is currently no other logical explanation for the differences in lesion locations between different equids.

In the Gariep CMZ the majority of affected animals had a single lesion and none of the animals had more than 4 lesions. Similar results were seen in a survey in Queensland where 22,2 % of horses affected by sarcoid had multiple lesions of which approximately 63 % had 2 lesions, 25.7 % had 3 lesions, 2.8 % had 4 lesions, 5.7 % had 5 lesions and 2.8 % had more than 5 lesions[13]. In the BNP outbreak, 7 (53.9 %) animals had a single lesion, 2 animals (15.4 %) had 2 lesions, and 4 animals each had 3, 5, 7 and 13 lesions, respectively[30]. The average number of lesions per horse in continental Europe is about 3 compared with 25–30 lesions per horse in the UK[9]Efficiency of vector transmission, climate, contact rate of vectors with infective material, host susceptibility, viral virulence, virus strain and numerous other variables could be responsible for differences in the number of lesions per individual.

Equine sarcoid has for many years been a difficult clinical entity to deal with in horses. In a free-ranging wildlife population, where minimal human interference is the ideal, it presents an even more difficult problem to deal with. Because genetic susceptibility to equine sarcoid is a strong possibility, the disease in CMZ should be researched most intensively in the cytogenetic field. The danger of offspring inheriting susceptibility to equine sarcoid can at the moment only be addressed by removing affected individuals from the gene pool. The reverse side of the problem is that the CMZ meta-population is already inbred[23] and the removal of individuals only exacerbates the problem. Unwillingness of certain conservation agencies to provide unrelated male breeding stock for introduction to inbred populations is also a problem. Ideally if one could identify female individuals which are genetically susceptible to the equine sarcoid and remove them to an isolated breeding area where they are cross-bred with non-susceptible males,

the population could over time be bred to be sarcoid resistant with improved genetic variability. It was found at the GNR and at the BNP[30] that treatment with autogenous vaccine, surgery and BCG (BCG vaccine SSI, Statens Serum Institute, Denmark), caused tumour regression in the majority of sarcoid-affected CMZ, which makes it possible to salvage affected individual for out-breeding projects. Not being able the identify genetically resistant and -susceptible CMZ is at this stage the most crucial shortcoming in managing this disease in the endangered Cape mountain zebra.

ACKNOWLEDGEMENTS

Sincere appreciation is expressed to the Free State Department of Tourism, Environment and Economic Affairs for allowing this study to be conducted and all the personnel who assisted with different aspects of the study.

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CHAPTER 4

DISCUSSION

4.1 INTRODUCTION

Although equine sarcoid as a disease entity in horses has only been described as recently as 1936[30], it may have been present in domestic equids much longer than this. With outbreaks as the recent ones in GNR and BNP, it can be regarded as an emerging disease in CMZ. The fact that the CMZ have been kept in conservation areas for many years without any cases having been reported, and the sudden emergence of outbreaks in two isolated populations at about the same time is difficult to explain. The only possible explanation is that inbreeding in these small CMZ populations reached a threshold at which some genetic deficit led to the population becoming susceptible to the development of equine sarcoid.

With the exception of the malevolent type[35], which has not been seen in the GNR, the clinical appearance of sarcoids in CMZ does not differ from that in domestic equids. As in horses, a histological confirmation is necessary to distinguish sarcoid from other differential diagnoses[35].

The histological appearance of sarcoids in CMZ is also similar to sarcoids in domestic equids. Histological diagnosis of sarcoid in CMZ is therefore reasonably easy. Recent advances in molecular biology and the availability of PCR diagnostic tests for BPV[3,19,89] can make confirmation of a histological diagnosis an inexpensive option to distinguish equine sarcoid from granulation tissue and from some other similar looking tumours.

4.2 PREVALENCE AND INCIDENCE

With the exception of the Bontebok National Park (BNP) population[42], the prevalence of equine sarcoid in the GNR CMZ population is significantly higher than that found in adjoining horse populations, Burchell's zebra *(Equus quagga burchellii*) populations on other reserves in the Free State as well as other CMZ populations in other parts of the country. This is a clear indication that there is some susceptibility factor at work in the CMZ population of

GNR. With the evidence of genetic susceptibility in horses[2,13,31,45,51,53,69], especially in the area of the MHC class I coding region[13,39,50], there is good reason to assume that some genetic susceptibility factor exists in the GNR CMZ population. This is also supported by preliminary genetic studies indicating that the GNR population has lost a significant amount of genetic diversity[77], which may have resulted in inbreeding depression. Inbreeding depression may then be expressed as an increased susceptibility to equine sarcoid. The overall prevalence of sarcoid for horses and donkeys in the northern hemisphere range between 2% and 7%[35] whereas the prevalence in the GNR CMZ increased from 9.4% in 1995-1996 to 24.4% in 2002-2003.

4.3 AGE AND SEX

Although sample size is often too small in a case study, there seems to be no significant sexual predilection for sarcoid development in the CMZ at GNR. Of the 39 cases, 19 were male and 20 female. The findings in the Queensland horse survey[51], and the sarcoid outbreak in CMZ of the BNP were similar[99]. Several other studies in horses and donkeys, however, reported an increased risk for young males. Geldings also seem to be more at risk than stallions [53,73, 74,75,91].

One should however be careful when interpreting risk as a parameter of predilection. Geldings (usually as young males) may be predisposed artificially as a result of the castration wound[35,81], and the possible attraction of vectors to the wound. Similarly, population composition may also cause apparent skewed gender susceptibility. It may also explain why the prevalence of sarcoid in geldings is higher than in stallions. Geldings, because they are preferred to stallions in sport and as working horses, make up a larger proportion of the male population than stallions.

4.4 PREDILECTION SITES AND MULTIPLICITY

From the literature, it is clear that there is a difference in the predilection sites between different locations and reference clinics. There are also indications that there is sometimes a difference in predilection sites between males and females[75]. In most of the studies in horses, the head and neck and the limbs are usually amongst the three most important sites of sarcoid

occurrence[7,51,75,84,87]. The inguinal and ventral abdominal areas, however, do stand out in certain studies as the more important sites of sarcoid occurrence[75,91].

In the Gariep CMZ population, the most important site in males was the inguinal area (55.2%) followed by the head (17,2%). In females, the head (41.4%) and then the inguinal area (27.6%) were the most important areas for sarcoid occurrence. The major difference between the species seems to be the absence of sarcoids on the lower limbs of the Gariep CMZ (only one case of the 39 had a lesion on the lower limb). This difference may be due to equestrian sports for which horses are often used. In most equestrian sports, some sort of protective padding is usually applied to the coronet, metacarpal and metatarsal areas. Especially with racing, jumping, polo and polo-crosse, there is an increased risk of injury to these sites. The injured area could attract vectors and may thus become infected more easily with BPV[35] with an increased risk of sarcoid development. The areas are also exposed to chronic irritation by the padding[81]. Possibly this explains the higher incidence of sarcoid lesions on the lower limbs of domestic horses.

It is interesting to note that in the sarcoid affected CMZ in BNP, the trunk (especially the ventral abdomen) and axillae are the areas most frequently affected[99]. The head only accounts for 11.4% of lesions in the BNP population[99] compared to 29.3% for the Gariep CMZ. The explanation for the differences between the BNP and GNR populations would be speculative at this stage. One possibility may be a difference in invertebrate vectors for the two locations and that these have different preferred biting sites (assuming that BPV is transmitted by a vector). Another may be the vegetation type. The thorny vegetation in the BNP may cause minor injuries to the axillae and ventral abdomen, leading to an increased local predisposition to sarcoid development. It may also be a combination of vector and habitat and even other factors that can lead to skin lesions. In horses this has been shown to be a risk factor for sarcoid development[35]. One can possibly conclude that areas of thin hairless skin (preferred vector biting sites) and areas predisposed to trauma (blood attracts possible vectors)[35] and chronic irritation[81] are the sites most at risk of sarcoid development. The fact that there are so many differences between locations, genders and reference populations may be an indication of the multi-factorial nature of sarcoid site predisposition and development[81].

Regarding the number of lesions per animal, the tendency in the GNR CMZ is similar to that in the southern hemisphere[7,51] horse studies where the majority of cases had a single lesion

and few animals had more than 4-5 lesions. This is not the case in the United Kingdom (UK) where multiple lesions are more common[35]. This difference could be a factor of the type of insect vectors and of the viral virulence and ease of transmission.

4.5 SARCOID TYPE

Of the six clinical types of equine sarcoid, as classified by Knottenbelt[35], only the malevolent type was not seen in the GNR sarcoid outbreak. The fibroblastic type sarcoid accounted for 60% of lesions in the Gariep sarcoid outbreak (Table 4.5.1). The absence of the malevolent type in the Gariep CMZ may only be a function of sample size.

TABLE 4.5.1: Classification of lesions (n=55) into macroscopic appearance according to Knottenbelt[35].

Sarcoid Type	Male	Male %	Female	Female %	Male + Female	Combined %
Fibroblastic unclassified	**4**	**14.81%**	**5**	**17.86%**	**9**	**16.36%**
Fibroblastic Type I	**9**	**33.33%**	**5**	**17.86%**	**14**	**25.45%**
Fibroblastic Type II	**4**	**14.81%**	**6**	**21.43%**	**10**	**18.18%**
Total Fibroblastic	17	62.96%	16	57.14%	33	60%
Nodular	**2**	**7.41%**	**4**	**14.29%**	**6**	**10.91%**
Verrucose	**5**	**18.52%**	**4**	**14.29%**	**9**	**16.36%**
Fibroblastic x nodular	**2**	**7.41%**	**0**	**0.00%**	**2**	**3.64%**
Verrucose x fibroblastic	**1**	**3.70%**	**3**	**10.71%**	**4**	**7.27%**
Mixed	**0**	**0.00%**	**1**	**3.57%**	**1**	**1.82%**
TOTAL	**27**	**100.00%**	**28**	**100.00%**	**55***	**100.00%**

*Some early lesions without proper classification of lesion type were not included in data.

The nodular type of sarcoid is most probably only a successional stage in the development of the fibroblastic type lesion. Initially, in the early stages of the lesion, the epidermis is intact (Figure 4.5.1).

Figure 4.5.1: Nodular sarcoid lesions on the prepuce of a CMZ.

As the uncontrolled fibroblasts and collagen increase in number and volume, the overlying epidermis is displaced (Figure 4.5.2, 4.5.3 and 4.5.4), exposing the raw fibroblastic lesion, which is often encrusted as the sero-cellular exudate dries (Figure 4.5.4). Mixed types are thus possibly only transformational stages of three basic types (fibroblastic, verrucose and malevolent).

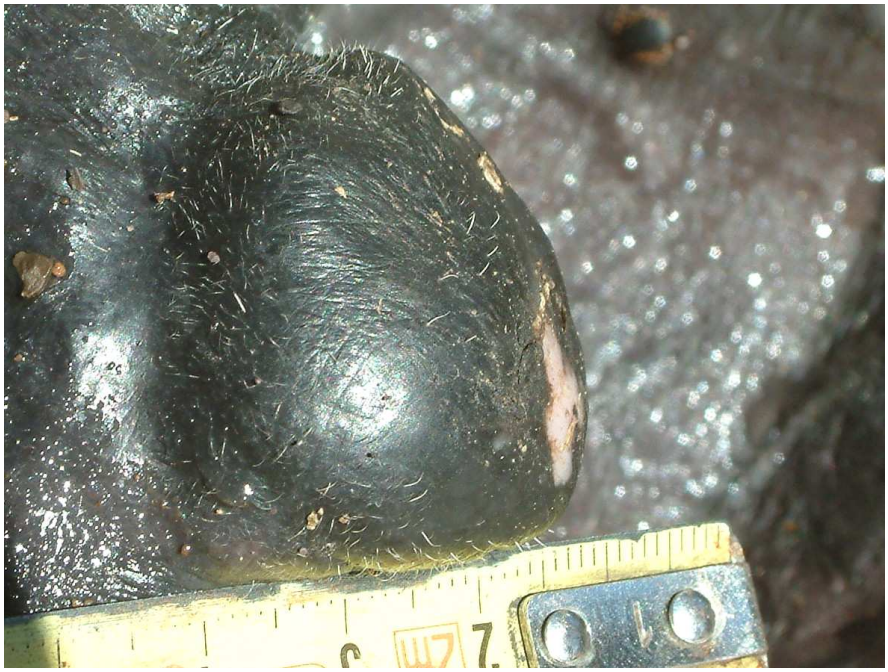

Figure 4.5.2: Nodular sarcoid with epidermal thinning and in the process of being displaced.

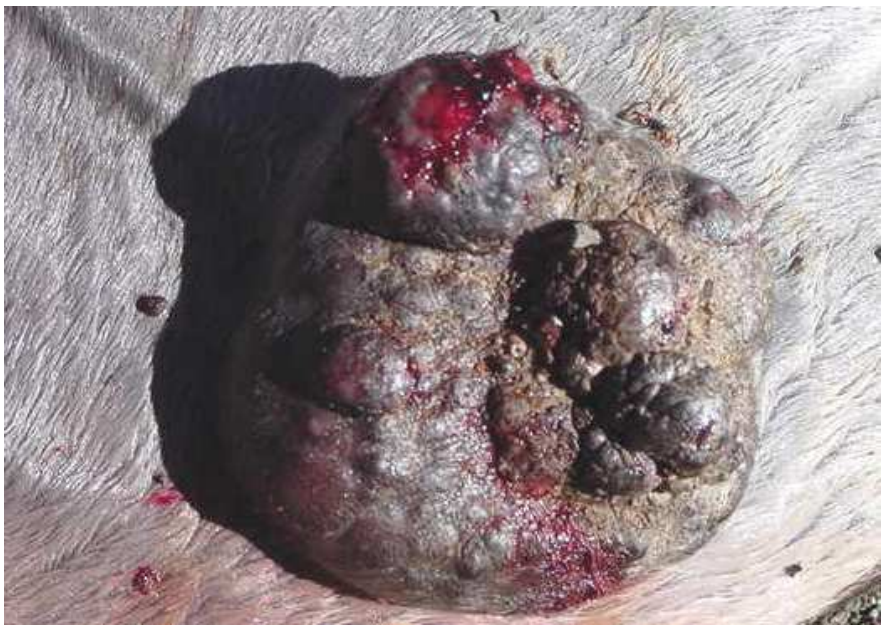

Figure 4.5.3: Nodular stage sarcoid with loss of epidermal component in some areas and in the process of developing into the fibroblastic type.

Figure 4.5.4: Fibroblastic sarcoid with remnants of the nodular stage.

One case of a possible mixed occult/verrucose sarcoid was seen in the GNR sarcoid outbreak (Figure 4.5.5). In horses this lesion type usually occurs around the mouth in the areas of soft hairless skin. They often progress to form the verrucose type[23,35]. The occult sarcoid recorded in the Gariep CMZ also occurred on the muzzle and had elements of the occult and verrucose types with areas of fibroblastic transformation (Figure 4.5.5).

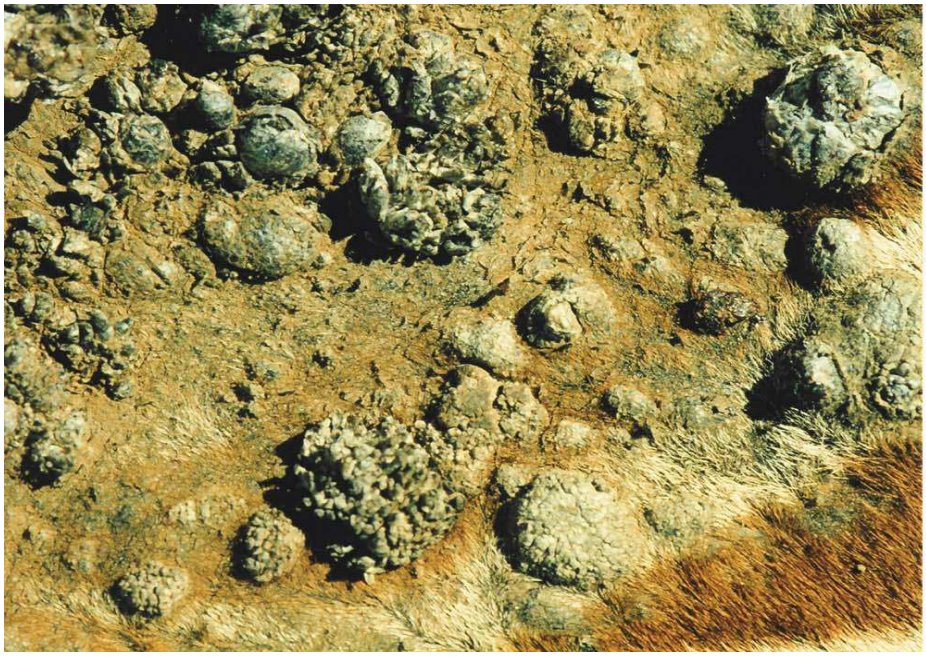

Figure 4.5.5: Mixed sarcoid on the muzzle of a CMZ**.**

Lesions can become large to the extent that they may limit the functionality of the animal in a mechanical manner (Figure 4.5.6 and 4.5.7) depending on the location of the lesion.

Figure 4.5.6: Large sarcoid lesions may impose mechanical limitations on the animal depending on the location and size of the lesion.

Figure 4.5.7: Large sarcoid lesion which could result in loss of fluid and protein from exudation and mechanical damage**.**

Affected CMZ can lose a substantial amount of proteinaceous fluid from large ulcerated fibroblastic lesions; to such an extent that hypoalbuminaemia develops. In a once-off observation in 2003, blood samples were collected from 15 sarcoid affected animals with lesions on different locations. A negative correlation was found between sarcoid size and albumin level (r=-0.76). The sample size was however too small for any concrete conclusions.

Thus as far as clinical appearance is concerned, the presentation of sarcoid in CMZ seem to represent that found in domestic equids[35], except for the malevolent type which was not seen in the Gariep CMZ; even in horses it is rare[35].

4.6 GROWTH RATE

In the literature no data could be found on the actual growth rate of sarcoids in domestic horses. The type of lesion may influence the growth rate of a sarcoid lesion. Treatment or injury may also affect growth rate. Some lesions tend to become more aggressive after certain treatments[35]. Even within the same lesion type, some lesions will progress very slowly, while

others are more aggressive. The form and location of the tumours on the body make it difficult to determine the weight or volume of lesions accurately. In CMZ, interference with the lesion (taking a biopsy or surgically removing one of multiple lesions) may cause an immune response that could interfere with the natural growth rate of the tumour. It is therefore important when growth rates of sarcoid tumours are studied, that the diagnosis be confirmed, retrospectively. In our study many of the CMZ received autogenous vaccine, which in some cases led to post-vaccination regression. The 5 of 22 animals that did not respond to the various treatment regimens had an average annual sarcoid-volume increase of 260% (volume index; see Chapter 3). This means that a lesion the size of a tennis ball, may have developed into a huge tumour within 2-3 years (Figure 4.5.6). Early detection of sarcoids is thus of utmost importance when treatment is considered as an option in CMZ.

One should opt for bi-annual visual examination of the CMZ herd for any suspect lesions if the salvage of affected animals is considered important. In practice, having a helicopter available specifically for the capture and treatment of affected individuals is often too expensive. Therefore one is mostly forced to combine treatment of sarcoid affected animals with an annual game capture or with aerial count operations.

4.7 TRANSMISSION

Although mechanical transmission may be possible in domestic horses because of stabling practices[20], it does not explain the occurrence and predilection of sarcoid tumours for the inguinal areas of CMZ at GNR. If it was transmitted mechanically from zebra to zebra, one would expect to see more lesions in contact areas or where wounds are inflicted by fighting (mostly the neck and lateral aspects of the hindquarters). According to Penzhorn wounds were located in the neck area and, in one case, on the tip of one ear after fights between stallions for dominance[65]. There was no predilection for sarcoid development at these sites in the Gariep CMZ. The transmission of the disease by insect vector or vectors seems to be the only logical conclusion. In horses in the UK Knottenbelt also mentioned that sarcoids, which did originate from an injury site, were usually located in thin-skinned, sparsely haired areas; the preferred areas of biting insects[35]. He also suggested the possibility of insect vectors being one of the modes of transmission. For a papillomavirus, which is not present in normal body secretions to be transmitted from cattle to cape mountain zebra without direct contact, the only alternative explanation would seem to be via an arthropod vector.

Ulcerated nodular and fibroblastic tumours are probably the main source of viral DNA for insect vectors. Viral DNA has been found in superficial skin swabs collected from sarcoid affected and in-contact horses as well as from face flies caught in the vicinity of sarcoid affected horses[10]. Open exudating sarcoids would attract insect vectors that could then be exposed to BPV-DNA in the fluids.

4.8 INBREEDING DEPRESSION

The GNR population increased from 9 animals in 1986 to 82 animals in 2003, which is equivalent to an average annual growth rate of 15.5% (27 animals were translocated and five animals introduced during this period). Compared to the national average of 9.6% for the period 1995-1998, this is an excellent population growth rate[56]. This growth rate was realized despite the effects of the sarcoid outbreak on the population (4 known sarcoid related mortalities, 10 sarcoid affected animals euthanased for humane reasons and 10 sarcoid affected animals presumed dead).

Apart from the sarcoid outbreak (which could reflect decreased disease resistance), inbreeding depression expressed as lower birth weight and survival of offspring, a lower reproduction rate and lower resistance to environmental stress[34], was not observed in the GNR population. Inbreeding depression does however not necessarily encompass all the factors mentioned above. Inbreeding depression, which is measured by comparing the fitness of progeny from outbred individuals to that of inbred individuals[34], may have been masked in the GNR population by favourable habitat that increases production above that of outbred benchmark populations subjected to less favourable habitats. Both the GNR and BNP populations (high sarcoid prevalence) are less heterozygous than the MZNP population[77] (low sarcoid prevalence). This may be an indication that inbreeding depression in the form of increased susceptibility to disease may be present in the two affected populations. Familial tendencies for high prevalence of equine sarcoid have been described in horses with highly inbred lineages[31].

If a similar outbreak of equine sarcoid occurred in a poor performing population like the one at GMNR, the effects could lead to extinction within just a few years. GMNR as one of the

three main founder gene pools of CMZ is extremely important from a genetic conservation point of view. The long-term probability of population growth in the GMNR is uncertain despite the fact that based on the current performance of the population; a population viability analysis did not predict extinction in the next 50 years[96]. Any factor, such as sarcoid, influencing population growth negatively could make the population vulnerable to extinction in the near future.

The fact that the sarcoid susceptibility status of many other CMZ populations is unknown is disturbing. The absence of sarcoid in a population does not mean that the population is necessarily resistant to the development of sarcoid. It could also be a reflection of the isolation of the population from BPV. It is imperative therefore that research is conducted as soon as possible to determine the gene/-s responsible for the increased susceptibility to equine sarcoid. With this knowledge, populations can be evaluated for sarcoid susceptibility risk and more accurate PVA's can be done to determine management actions to be taken to improve viability and fitness of the *Equus zebra* species.

There is a possibility that CMZ are generally more susceptible to some diseases than domestic horses. This statement is based on the serious outbreaks of equine sarcoid in the GNR and BNP and the possible beginning of another outbreak in the more outbred population of the MZNP. As mentioned before, the fact that some populations of CMZ are as yet unaffected by sarcoids may be the result of their isolation from cattle and hence a source of BVP. If this is the case, the long-term survival of the species could be at risk. This necessitates the need for intensification of research into the genetics of the disease.

There is of course the possibility, that historically CMZ had an inherent poor resistance to the development of equine sarcoid, even when the population still had a healthy genetic variability, because they had never been exposed BPV. Cattle pastoralists did not become established in southern Africa until ad 1000 (Microsoft ® Encarta ® Premium Suite 2005. © 1993-2004 Microsoft Corporation). Thus it is possible that CMZ did not evolve in the presence of BPV, and are therefore more susceptible than domestic equids who have had a longer exposure, and time for co-evolution with BPV than CMZ have had. Burchell's zebra do not seem to be more susceptible to the disease than domestic equids. Even in areas of eastern and southern Africa where cattle use the same pastures as zebra, no outbreaks of equine sarcoid have been documented.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 GENERAL

This is a study of an outbreak of equine sarcoid in the CMZ of the GNR with severe consequences. Another outbreak of similar proportions occurred in the BNP□42. Even with the current incomplete knowledge of equine sarcoid (especially the genetic aspects of the disease) in CMZ and in domestic equids, there are assumptions and deductions that could at this stage be used and applied to manage the problem in CMZ until genetic susceptibility markers have been identified. The important facts and deductions about equine sarcoid in CMZ can be summarized as follows:

- Equine sarcoid as a disease entity in CMZ is similar to that in domestic equids as far as aetiology, epidemiology and clinical and pathological appearance is concerned.
- The sarcoid prevalence in some CMZ populations may be an indication of an excessive susceptibility to the disease when compared to domestic horses and to more out-bred Burchell's zebra populations.
- Inbreeding is a possible risk factor.
- Inbreeding should be prevented by mixing genes from the three main founder gene pools on a continuous basis (meta-population genetic management).
- Un-affected CMZ populations may not necessarily be resistant to the disease.
- Some gene mutation or loss of allele/-s is most likely responsible for increased sarcoid susceptibility in CMZ.
- A "susceptibility gene" could be inherited. It has been suggested that with evidence of certain breeds with certain ELA antigens being more susceptible to sarcoid, that these Class I ELAs could be used as markers to identify sires which should not be used for breeding[39]. The authors suggested that the mere presence of sarcoid tumours should disqualify a sire for breeding purposes[39]. In the absence of a marker for genetic susceptibility in CMZ, affected animals should be isolated and prevented from breeding with un-affected animals or they should be culled.
- Such a "susceptibility gene" is probably recessive.
- The disease has to be resolved on a meta-population basis and not by treating individual cases.

- Females with a recessive homozygous gene mutation for sarcoid susceptibility may possibly be of use in out-crossing breeding projects once the link is established between sarcoid susceptibility and the responsible gene mutation/deficit. It therefore makes sense not to exterminate these animals from the meta-population.
- Older unaffected individuals which have been present in a population with a high sarcoid prevalence for a number of years are most likely to be resistant to sarcoid and could be used (in the absence of genetic markers for sarcoid susceptibility) for out crossing purposes on affected females.
- That sarcoid-affected males are of less value and culling should be considered

5.2 OBSTACLES TO THE MANAGEMENT OF EQUINE SARCOID IN CMZ

Some problems that may present an obstacle to the effective management of equine sarcoid in the CMZ meta-population are:

- Some governmental conservation bodies seem to be inclined to keep fragmented gene pools separate because of phenotypic differences that have developed in these small populations as a result of isolation. The impression created is that these institutions want to guard their populations as unique. As such this will eventually be to the detriment of CMZ conservation.
- The absence of a genetic marker that can be used to identify sarcoid susceptible individuals.
- The absence of clear guidelines on the management of a sarcoid outbreak in a CMZ population. This may be the result of insufficient understanding of the genetic basis of the disease and many other factors affecting the epidemiology of the disease.
- Finding locations isolated from sarcoid resistant populations where sarcoid susceptible CMZ (affected animals that recovered after treatment) can be used in out-breeding projects.

5.3 ACTIONS NEEDED FOR MORE EFFICIENT MANAGEMENT OF THE SARCOID PROBLEM IN CMZ

- An effective National CMZ Management plan aimed at conserving the genetic viability of the meta-population is needed. This management plan should include contingency guidelines to deal with sarcoid outbreaks in previously sarcoid-free CMZ

populations. A management committee consisting of representatives of parties involved in CMZ conservation has already been established after a workshop held at Gamka Mountain Nature Reserve in 1998. This is definitely a positive step towards coordinating conservation and meta-population management of the CMZ.

- Identification of the gene/-s responsible for sarcoid susceptibility is urgently needed.
- Identification of susceptible populations should be conducted once the genetic aspects have been elucidated.
- Establishment of sarcoid resistant populations using individuals from all three founder gene pools.
- Management to increase heterozygosity (mixing the three gene pools). This should be done taking cognisance of the social habits of CMZ and of the demographics of breeding herds. It may be necessary to use females from one lineage only and introduce males from another lineage in order to ensure that forced cross-breeding takes place and to ensure that it happens rapidly.
- Investigating the possibility of out-crossing sarcoid affected females to sarcoid resistant males in order to incorporate sarcoid susceptible individuals into the meta-population. Affected animals may have other valuable genes necessary to ensure long-term genetic viability.
- Research into treatment methods to salvage sarcoid affected females for above-mentioned purpose.
- Research into sarcoid prevention if susceptible populations are to be used for out crossing breeding projects.

In conclusion, it is clear that equine sarcoid may pose a definite danger to the long-term survival of the endangered CMZ. If actions are not taken to address the root causes of the disease and manage it accordingly, equine sarcoid may become a factor that could in future be responsible or partly responsible for the extinction of the species. The fact that individual animals may be salvaged effectively by some of the treatment methods used in horses, does not provide the solution to the disease in free-ranging CMZ populations. The basis of the solution should be the restoration of the natural resistance of the CMZ meta-population to the disease.

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ANNEXURES

APPENDIX 1

DATA CAPTURE FIELD FORM

CAPE MOUNTAIN ZEBRA – GARIEP NATURE RESERVE

Date..... Age..... Sex:.....
Ear notch number:..... Ear tag colour:..... Ear tag number:.....

Samples:

EDTA Serum Sarcoid in Formalin

Sarcoid frozen Normal skin frozen Skin in formalin □

Lesions:

Lesion 1: Length (cm).....width (cm):.....height (cm)..... Type:.....

Lesion 2: Length (cm).....width (cm):.....height (cm)..... Type:.....

Lesion 3: Length (cm).....width (cm):.....height (cm)..... Type:.....

Lesion 4: Length (cm).....width (cm):.....height (cm)..... Type:.....

Comments:.....

.....

Photos:

.....

Treatment:

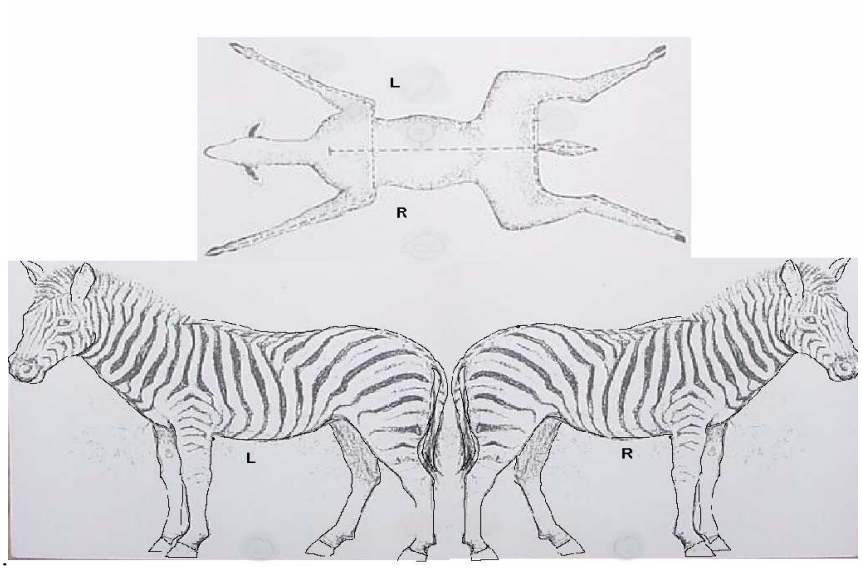
.....

.....

Comments:

.....

Lesion location:



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APPENDIX 2

GLOSSARY OF TERMS

Allele:

The term allele is used for two or more alternative forms of a gene resulting in different gene products and thus different phenotypes. In a haploid set of chromosomes there is only one allele at its specific locus. Diploid organisms have 2 alleles at a given locus, i.e. a normal and a mutant allele. A single allele for each gene locus is inherited separately from each parent (e.g., at a locus for eye colour the allele might result in blue or brown eyes). An organism is homozygous for a gene if the alleles are identical, and heterozygous if they are different[78].

Allogenic:

Genetically different but of the same species, used to describe tissues that are genetically different and therefore incompatible when transplanted[78]

Apoptosis:

Cell death. During apoptosis, the cell shrinks and its surface membrane takes on a characteristic "boiling" appearance with the formation of blebs and pinched off vesicles. Chromatin, the genetic material within the nucleus, condenses and the cell's DNA is cut up by enzymes called nucleases. Finally, the whole cell collapses into membrane-bound apoptotic cell fragments that are rapidly "eaten" by neighbouring cells via the process of phagocytosis. Apoptosis is therefore both an efficient way of killing unwanted or damaged cells and of packaging the remnants for rapid removal. (Microsoft ® Encarta ® Premium Suite 2005. © 1993-2004 Microsoft Corporation. All rights reserved.)

Autogenous:

Vaccine: made from organisms collected from a specific disease outbreak / -graft: taken from one part of a patient's body and transferred to another

Autologous transmission:

Transmission from one site in/on an individual to another location in/on the same individual. Autologous: Tissue from the same individual[78].

Cross breeding:

Breeding method using directed crossing to generate higher variability[78]

Episomal:

Independent genetic element: a genetic unit that can multiply independently in host cells or when integrated with a chromosome. Bacterial plasmids are examples of episomes[78].

Gene flow:

The exchange of genes between different but (usually) related populations[78]

Gene:

A gene is an ordered sequence of nucleotides located in a particular position (locus) on a particular chromosome that encodes a specific functional product (the gene product, i.e. a protein or RNA molecule). It includes regions involved in regulation of expression and regions that code for a specific functional product[78].

Genetic diversity/variation:

A property of a community of organisms of a certain species, in which members of the community have variations in their chromosomes due to a large number of slightly dissimilar ancestors; this property makes the community in general more resistant to diseases or to changing ecological conditions[78].

Genetic drift:

The random changes that occur in the gene frequency of small, isolated populations, resulting in the loss or preservation of certain genes over the generations[78].

Haplotype:

A set of closely linked genetic markers present on one chromosome which tend to be inherited together (not easily separable by recombination). Some haplotypes may be in linkage disequilibrium[78].

Heterozygosity:

The presence of different alleles of a gene at one or more loci. Cf. heterozygote[78].

Homologous chromosomes:

Chromosomes that pair during meiosis; each homologue is a duplicate of one chromosome from each parent.

Inbreeding depression:

A loss of vigour or yield due to inbreeding[78].

Inbreeding:

The mating of genetically related individuals. Mating between relatives. Breeding through a succession of parents belonging to the same stock[78].

Incidence:

In disease epidemiology, the incidence is the number of newly diagnosed cases during a specific time period. The incidence is distinct from the prevalence that refers to the number of cases alive on a certain date. / the frequency of new occurrences of disease within a defined time interval. Incidence rate is the number of new cases of a specified disease divided by the number of people in a population over a specified period of time, usually one year.

Mutation:

An abrupt change of genotype which is inherited. Any permanent and heritable change in DNA sequence. Types of mutations include point mutations, deletions, insertions, and changes in number and structure of chromosomes. (Cf. polymorphism) [78].

Oncogene:

A gene, one or more forms of which is associated with cancer. Many oncogenes are involved, directly or indirectly, in controlling the rate of cell growth.

Open reading frame (ORF):.

A sequence of DNA following an initiation codon that does not contain a stop codon. Detection of an open reading frame in DNA implies the presence of a gene that codes for a protein[78].

Outbreeding/Allogamy:

Breeding between unrelated individuals of the same species. Some species support or force allogamy by complex mechanisms, such as genetic self-incompatibility, heterostyly, dichogamy or herkogamy. Also called xenogamy, outbreeding, and sometimes cross breedinghomologous transmission

Polymerase chain reaction (PCR):

A method, developed, for amplifying a DNA base sequence using a heat-stable polymerase and two 20-base primers, one complementary to the (+)-strand at one end of the sequence to be amplified and the other complementary to the (-)-strand at the other end. Because the newly synthesized DNA strands can subsequently serve as additional templates for the same primer sequences, successive rounds of primer annealing, strand elongation, and dissociation produce rapid and highly specific amplification of the desired sequence. PCR also can be used to detect the existence of the defined sequence in a DNA sample (see DNA amplification fingerprinting. Several variations have been developed for specific needs. May be combined with reverse transcription of mRNA to cDNA to amplify an mRNA so called RT-PCR[78].

Polymorphism:

Difference in DNA sequence among individuals. Applied to many situations ranging from genetic traits or disorders in a population to the variation in the sequence of DNA or proteins. Genetic variations occurring in more than 1% of a population would be considered useful polymorphisms for genetic linkage analysis. Compare mutation[78].

Population:

A group of organisms of the same species relatively isolated from other groups of the same species[78].

Prevalence:

Prevalence is a statistical concept referring to the number of cases of a disease that are present in a particular population at a given time.

Stochastic:

Stochastic Process, process in which a system changes in a random manner between different states, at regular or irregular intervals, Examples are genetic drift, disease outbreaks and natural catastrophes[78]

THE END