Prevalence and body distribution of sarcoids in South African Cape mountain zebra (*Equus zebra zebra*)

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ABSTRACT

There are no reports in the literature describing any tumours, and specifically sarcoids, in zebras. The equine sarcoid, a locally aggressive, fibroblastic skin tumour, is the most common dermatological neoplasm reported in horses. The Cape mountain zebra (CMZ) has been described as one of the most vulnerable mammals in South Africa with current populations existing in isolated units. All South African CMZ are descendants from no more than 30 individual animals originating from 3 populations, namely the Mountain Zebra National Park, and Kammanassie and Gamka Mountain Nature Reserves near Cradock. The possibility therefore exists that the existing populations arose from a very small gene pool and that they are considerably inbred. A reduction in major histocompatibility complex diversity due to genetic bottlenecks and subsequent inbreeding probably contributed to uniform population sensitivity and the subsequent development of sarcoid in two CMZ populations, namely in the Bontebok National Park and Gariep Nature Reserve. The entire population of CMZ in the Bontebok National Park was observed and sampled during 2002 to document the prevalence and body distribution of sarcoids. During the same year, a comparative study was carried out on an outbred population of Burchell's zebra in the Kruger National Park. The prevalence in CMZ in the Bontebok National Park was 53 %, while the Burchell's zebra in Kruger National Park had a prevalence of 1.9 %. The most common sites for sarcoid in CMZ were the ventral abdomen and limbs. Prevalence of sarcoids in horses recorded in the literature varies between 0.5 % and 2 %. The Gariep Nature Reserve recently reported a prevalence of almost 25 % in CMZ in the reserve.

Key words: head, bodily distribution, Bontebok National Park, Cape mountain zebra, Gariep Nature Reserve, prevalence, sarcoid.

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INTRODUCTION

Sarcoid is worldwide in distribution and comprises the most common tumour in the horse ^{15,25}. The prevalence of sarcoid in horses has been well studied and ranges from 0.5 to 2.0 % ^{11,15}. Most reports suggest that there is no apparent gender, coat colour, seasonal, geographic or breed predilection for the occurrence of sarcoid in horses ^{9,27}. A higher incidence of sarcoid has been suggested in Quarter horses, Arabians and Appaloosas ¹⁹, while in Standardbreds it is purportedly lower. In addition, an increased occurrence of

sarcoid tumours has been recognised in certain families of horses¹⁵.

Researchers have identified an association between susceptibility to sarcoid and certain heritable cell-surface proteins, called major histocompatibility antigens. The major histocompatibility complex (MHC) is a group of genes that code for proteins involved in the immune response as well as protein components of the compliment system. The MHC comprises 3 different classes of genes with different locations and different functions. Class II genes have been implicated in susceptibility to sarcoid tumours^{4,5,18}. This class of genes codes for proteins found on the surface of lymphocytes and macrophages which act as receptors for foreign antigens. These receptors are responsible for the presentation of bound antigen to cells of the immune system³⁰. Class II MHC genes that encode particular equine leukocyte antigen (ELA) haplotypes, including ELA W13, have been associated

with increased susceptibility to sarcoid tumours ^{4,10,14}. Sarcoid tumours are fibroblastic in origin and have been reported to occur most commonly on the legs, ventral trunk and head of Equidae^{24,29}. Sarcoids have a reputation for being notoriously difficult to treat due to their locally infiltrative growth pattern, their simultaneous occurrence at multiple sites and their localisation at sites which compromise excision^{16,28}. In addition, they show high rates of recurrence after treatment or excision^{24,31}.

The Cape mountain zebra (Equus zebra zebra) has been described as one of the rarest mammals in South Africa and in the world²³. There are around 1500 animals remaining in populations, which are spread among various national parks, reserves, zoos and private reserves (SP Sasidharan, Faculty of Veterinary Science, Onderstepoort, pers. comm., 2005). All South African Cape mountain zebra (CMZ) are descendants from no more than 30 individual animals originating from 3 populations namely those at Mountain Zebra National Park (MZNP), and Kammanassie and Gamka Mountain Nature Reserves near Cradock³. All 3 of these populations had been confined to fenced areas for many generations and it is therefore likely that they are considerably inbred today.

A recent study was undertaken to document the degree of inbreeding in CMZ in Bontebok National park (BNP) and Gariep Nature Reserve (GNR). Domestic horse microsatellites were used to obtain allelic information. These two populations were compared to populations of CMZ without any history of sarcoids. The sarcoid affected populations had the lowest level of heterozygosity and polymorphism, thereby confirming their limited genetic diversity²⁶.

The current CMZ population in South Africa occurs in isolated units. Approximately 350 animals are held in the MZNP. Seeded populations from MZNP held in Bontebok National Park, Karoo Nature Reserve, and Gariep Nature Reserve, are currently estimated at 19 375 and 70 animals, respectively. The only original populations of CMZ still persisting are

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believed to be in Gamka Mountain Nature Reserve, Cradock, and Kamanassie Parks (B L Penzhorn, Faculty of Veterinary Science, Onderstepoort, pers. comm., 2004). There are other minor seeded populations, which originated from established populations such as the MZNP herd or from other original populations, such as the 130 CMZ in Commando Drift Nature Reserve, near Cradock.

High morbidity due to sarcoid tumours has been reported in the CMZ of the BNP and GNR. No studies have previously been conducted to determine the prevalence and body distribution of sarcoids in the CMZ, despite these reports. A few mortalities, due to severe lameness, have even been attributed to this condition. In this paper the prevalence and body distribution of sarcoids in the CMZ of BNP are reported and compared with CMZ in GNR and an outbred population of BZ in

MATERIALS AND METHODS

During the study, the entire population of CMZ in the BNP was investigated. The sarcoids included in this study were tentatively identified based on their gross morphology according to Pascoe and Knottenbelt²² and subsequently all confirmed by histopathology (to be reported in another paper).

The BNP is situated in the foothills of the Langeberg Mountains, located in the region of Swellendam. The park was proclaimed in 1931 to prevent the last few remaining Bontebok from becoming an extinct species. The region has a temperate climate with an average rainfall of about 500 mm per annum, occurring mainly during early summer and winter and is also one of the largest remaining 'Renosterveld islands' containing several plant species found nowhere else in the world. The 2786 hectare park is bordered to the south by the Breede River and provides a refuge for species like Bontebok, CMZ and Red hartebeest. Coordinates for BNP are longitude 20°06.01.36'E and latitude 34°33.01.11'S.

All CMZ in BNP were immobilised during 2002. A total of 15 CMZ was present in the park, consisting of 9 females (6 subadults and 3 adults) and 6 males (3 subadults and 3 adults). All animals were immobilised from a helicopter using a combination of 4–7 mg etorphine hydrochloride (M99® Novartis, Isando), 40–80 mg azaperone (Azaperone® Bayer-Animal Health, Isando) and 1500 i.u. hyaluronidase (Hyalase® Kyron Laboratories, Benrose). Once the animals were down, a ground team moved in and covered their eyes and blocked the ears. All animals were identified by microchip

Table 1: Prevalence of sarcoids in the Cape Mountain Zebra and Burchell's zebra in the respective parks during 2002.

Park or Reserve	Total number of zebra observed in 2002	Prevalence	Affected animals with multiple tumours		
BNP (CMZ)	15	8 (53 %) ^{a,b}	4 (50 %)		
GNR (CMZ)	77	19 (24,7 %) ^{a,c}	14 (73.7 %)		
KNP (BZ)	104	2 (1.9 %) ^{b,c}	0		

BNP = Bontebok National Park; GNR = Gariep Nature Reserve; KNP = Kruger National Park; CMZ = Cape Mountain zebra: BZ = Burchell's zebra.

and the markings on either side were recorded by means of digital photography. After a thorough examination, the presence and distribution of tumours was recorded on a body chart. Sizes and appearance were recorded by means of digital photography. Distribution was divided into 3 anatomical locations: head and neck, ventral abdomen and limbs. Reversal of anaesthesia was done with 8–14 mg diprenorphine HCL (M50-50® Novartis, Isando).

The observations of the CMZ in the GNR is described by Nel *et al.*²¹. For the prevalence of sarcoid the 2002 survey, where each of the 77 CMZ was examined, was used. For the distribution of sarcoids on the body all cases observed in the GNR from 1996 to 2003 were used.

A control outbred population of 104 randomly selected Burchell's zebra (BZ) with an expected lower prevalence of sarcoids were observed in the Kruger National Park (KNP) over a period of a week. To avoid the possibility of observing the same zebras the location was changed from day to day. The animals were observed with binoculars at close range on bare ground or in short grass to record any sarcoid-like growths. They were grouped according to sex and approximate age (male, female, foals, subadults, adults). The presence of tumours and distribution on both sides of each animal were recorded.

Statistical analysis

Fisher's exact test for proportions was used to analyse the 2002 prevalence data of the 3 parks (Table 1). To compare median number of sarcoids on the head and neck, ventral abdomen and limbs of the CMZ in the BNP and GNR the Kruskal-Wallis test was used (Table 2).

RESULTS

Prevalence of sarcoids

The prevalence results between the 3 parks are summarised in Table 1. Eight of 15 (53 %) of the CMZ population at BNP had tumours. Of the affected animals, 4 of

8 (50 %) had multiple tumours. Two of the 104 (1.9 %) BZ observed had growths that looked similar to the sarcoids seen in the CMZ. The prevalence was higher in the BNP than the GNR (P=0.034) and both the BNP and GNR CMZ had a much higher prevalence than the BZ of the KNP (in both cases P<0.001).

Distribution on the body

Of the 8 sarcoid cases found in the BNP, 1 CMZ (12.5 %) had lesions on the head and neck, 6 (75 %) on the ventral abdomen and 4 (50 %) on the limbs (Fig. 1). The combined CMZ data from the BNP and GNR revealed a lesion predilection of 40% (19 of 47) on the head and neck, 81%(38 of 47) on the ventral abdomen and 25.5 % (12 of 47) on the limbs (Table 2). There was a statistically significant difference in overall prevalence between the 2 parks, but no difference in body location prevalence between sexes. However, there was a tendency for a greater prevalence of tumours in BNP on the limbs (P = 0.0618) and a significantly greater prevalence of tumours in BNP than GNR on limbs plus the body (P = 0.0418).

Of the two BZ observed in KNP 1 (50 %) had 1 lesion on the head and neck, while the other one (50%) had a lesion on the abdomen. No lesions were found on the limbs (Fig. 1).

DISCUSSION

There is evidence that the development of sarcoids in horses may be associated with a genetic predisposition¹. The CMZ in BNP originated from the MZNP, whereas the animals in GNR all came from a founder population numbering 6 animals originating in the Cradock area. It is likely that these 2 populations went through a genetic bottleneck in recent years. Research has shown a strong association between risk of sarcoid development and certain alleles of the Class II region of the equine MHC⁷. A possible reduction in major MHC diversity due to genetic bottlenecks and subsequent inbreeding may have contributed to uniform population sensitivity and the

^aPrevalence of sarcoids in BNP higher than GNR (P = 0.034).

^bPrevalence of sarcoids in BNP higher than in BZ of the KNP (*P* < 0.001).

[°]Prevalence of sarcoids in GNR higher than in BZ of the KNP (P < 0.001).

Table 2: Numbers of sarcoid affected Cape Mountain Zebra and numbers of sarcoids on the head and neck, ventral abdomen, limbs and combined ventral abdomen and limbs in the Bontebok National Park (2002) and Gariep Nature Reserve (1996–2003).

Park/reserve and period	Total sarcoid affected animals	Head and neck		Ventral abdomen		Limbs		Combined ventral abdomen + limbs	
		Animals	Lesions	Animals	Lesions	Animals	Lesions	Animals	Lesions
BNP (2002) GNR (1996–2003)	8 39	1 (12.5 %) 14 (35.9%)	2ª 18ª	6 (75 %) 25 (64.1%)	9ª 32ª	4 (50 %) 8 (20.5%)	5 ^b 8 ^b	8 (100%) 30 (76.9%)	14° 40°

BNP = Bontebok National Park: GNR = Gariep Nature Reserve.

subsequent development of sarcoid in these 2 CMZ populations. The recent study by Sasidharan²⁶ showed specific heterozygote deficiency within the 2 populations of the BNP and GNR. This is clear evidence that the genetic diversity of these small and isolated populations of CMZ has been reduced, possibly accounting for the high prevalence of 53 % and 25 % of sarcoid tumours observed, in the 2 parks respectively.

Although isolated cases of sarcoid have been diagnosed in BZ, there have been no confirmed reports in the KNP population. Our observations on random groups of BZ revealed a prevalence of 1.9 % of sarcoid-like growths. This is similar to the prevalence found in horses worldwide, which are assumed to be relatively outbred. The prevalence in our observed population of BZ may even be lower due to the fact that some or all of these growths that were recorded may be normal warts/papillomas on the skin or abnormal connective tissue after injury.

The head, limbs and abdomen were the most common sites for sarcoids in this study. Jackson¹² reported the predilection sites for equine sarcoids as the lower portions of the limbs, especially the metacarpal area, the head, particularly the eyelids and lips, and the prepuce. Three other studies are in agreement and found the most frequent sites for sarcoid to be the legs, ventral trunk and head (around eyes, pinnae, and commissures of lips)^{6,20,29}. A retrospective review of 99 studies, representing 662 sarcoids found the following relative predilection sites: legs, 45.8 %; head and neck, 31.6 %; chest and trunk, 8.8 %; abdomen and flank, 6 % and prepuce, $3.6\%^{29}$.

Statistically the prevalence of sarcoid was higher in BNP CMZ than in the GNR. The significance of this finding should be viewed with caution, however, because the GNR population was manipulated following the outbreak of sarcoid in 1995. A total of 13 sarcoid affected and 22 non-affected CMZ were removed from the Reserve from 1996 to 2001.

The CMZ of BNP had the highest number

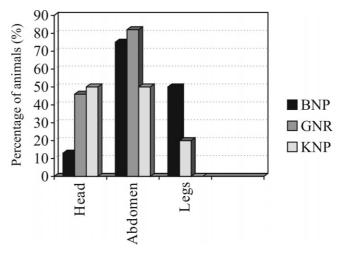


Fig. 1: Body distribution of sarcoids in Cape mountain zebra in the Bontebok National Park (2002) and Gariep Nature Reserve (1996–2003). Although the distribution of the Burchell's zebra are 50 %, note must be taken that the prevalence was only 2 animals out of 104.

of sarcoids on the ventral abdomen and legs, whereas in the GNR the prevalence was also highest on the ventral abdomen, but was followed by the head and then the legs²¹. The combined ventral body parts (ventral abdomen plus limbs) of CMZ in the BNP had a significantly higher number of sarcoids than in the GNR Table 2; P = 0.0418). This could be partly ascribed to the different vegetation types occurring in the 2 reserves. The vegetation in the BNP consists mainly of 2 key groups, namely Fynbos and coastal Renosterveld, although the latter is only found in limited areas of the reserve. A specific feature of the Fynbos in this reserve is the high prevalence of prickly bush (Cliffortia ruscifolia). This sturdy shrub has needle-like leaves that can easily traumatise the skin. In horse sarcoid, lesions often occur at wound sites or sites that are predisposed to trauma. Sarcoids may even appear in freshly healing wounds in previously unaffected horses or re-occur at the same site following apparent complete surgical removal of sarcoids up to 10 or more years later 12,22,29 Another author also reported an increased incidence of tumour development 3 to 8 months after the healing of a wound²⁴. In the BNP the legs of CMZ are continually exposed to trauma by the

prickly bush, which may explain the high frequency of lesions on the legs. The GNR habitat is mainly Nama Karoo with the dominant vegetation consisting of grassy, dwarf shrubland interspersed with grasslike Tassel Bristlegrass (Aristida congesta) and Lehmann's Lovegrass (Eragrostis lehmanniana). On the stony plains, Kapokbush (Eriocephalus ericoides), Silverkaroo (Plinthus karooicus) and Perdekaroo (Rosenia humilis), amongst many other shrubs, are common. They are unlikely to inflict trauma to the skin. The small population and thus sample size in the BNP is a potential source of bias and precludes firm conclusions being drawn. The study was also not designed to prove causality and the influence of habitat on the relative body distribution of sarcoids thus remains purely speculative. It is important to note here that the study in Bontebok was done during a single year, while the study in GNR was performed over a period of 8 years. The data collected in GNR can further be influenced by the fact that animals with sarcoids were removed from the population over the study period. Some authors have also pointed out that the limbs of horses are apparently more frequently affected in warmer climates (e.g. Australia and the southern United States), than in European coun-

^aNo difference between prevalence of tumours on the head and neck and ventral abdomen between the two parks (P = 0.2766 and 0.4224, respectively).

^bThere was a tendency for a greater prevalence of tumours in BNP on the limbs (P = 0.0618).

^cSignificantly more tumours on combined ventral body parts in BNP than GNR ($\dot{P} = 0.0418$).

tries 15 . The 25.5 % limb distribution in our study thus supports the assertion of these authors 17,22 .

Bastianello² differed slightly from Jackson¹² and reported that the head, particularly the ear, followed by the trunk and limbs was the most common site for sarcoids. In another study of 1044 sarcoids, 39 % lesions were found on the head and neck, 26 % on the body and 35% were on the legs¹⁹. Although, compared with BNP, the CMZ in GNR had a higher frequency of sarcoid on the head and neck; most authors agree that sarcoids can appear on any part of the body^{19,24,29}.

Sarcoids, arising at multiple sites, are relatively common 24 . The incidence of multiple tumours in horses varies from 14 % to 84 % 8,13,24 . This is similar to our study where we found 50 % and 36 % of CMZ with multiple sarcoids in BNP and GNR, respectively.

This study confirmed the high prevalence of sarcoids in CMZ in the 2 reserves in South Africa. We suspect that the inbred nature of these animals contributes to this high prevalence and suggest that further genetic studies are needed to confirm this.

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