CHAPTER FIVE:

MHC CLASS II VARIATION AMONG ZEBRAS WITH SARCOID TUMOURS

Part of this chapter was published in: *Journal of Equine Veterinary Science*. 2009. 29:320.

ABSTRACT

The endangered Cape mountain zebra (*Equus zebra zebra*) are protected in a few small isolated populations in South African game parks. Because of the small numbers, the herds are prone to inbreeding and development of hereditary problems. In 1995, sarcoid lesions appeared on zebras in two parks, affecting up to 24% and 53% of the populations in each park. This is much higher than the less than 1% prevalence among horses. Certain major histocompatibility complex (MHC) haplotypes are associated with increased risk for sarcoid tumours in horses. This study was undertaken to investigate whether or not zebras in these parks had become inbred for the MHC region with increased prevalence of a haplotype, conferring increased risk for sarcoid tumours. Single strand conformational polymorphism was used to assess genetic variation at the MHC among these zebras. Twelve sarcoid affected and 12 controls from herds where the disease occurred as well as 10 controls from zebras in parks where no sarcoid has been observed before, were tested. The results demonstrated that genetic variation existed for the MHC class II genes in these zebras and no haplotype was found associated with the presence of sarcoid tumours.

Keywords: MHC, sarcoid, Cape mountain zebra, polymorphism

5.1 INTRODUCTION

The Cape mountain zebra (*Equus zebra zebra*) was one of the rarest mammals in the world (Penzhorn 2003) and classified as vulnerable on the International Union for the Conservation of Nature and Natural Resources (IUCN) Red List 2009 (http://www.iucnredlist.org). They were saved from the brink of extinction and are protected in small numbers in a few isolated populations in South African game parks in their natural habitat. Although the numbers slowly increased in recent years, the genetic diversity and the continued existence of Cape mountain zebras are of great concern as the uneven distribution of a few relatively large populations makes them still vulnerable (Novellie *et al*. 2002).

Cape mountain zebra herds in South Africa had been confined in fenced areas for many generations and the opportunity of high inbreeding as a result of the non-territorial social organization of Cape mountain zebra occurred (Penzhorn & Novellie, 1991). For instance the population increase to a high density in a short period of time and cases have been reported where a young stallion became the herd stallion of his maternal herd (Penzhorn & Novellie, 1991). In 1995, equine sarcoid-like lesions (24.7% of animals) appeared in Cape mountain zebras from the Gariep Dam Nature Reserve (Free State Province) (Nel *et al*. 2006) and in 1998 in the Bontebok National Park (Western Cape Province) (53% of animals) (Lange 2004). An isolated case of a zebra that was euthanized due to the severity of sarcoid lesions was reported in 2004 in the Mountain Zebra National Park (Eastern Cape Province) (personal communication: Dr Dave Zimmerman).

The term “equine sarcoid” was first used by Jackson in South Africa in 1936 to describe a distinctive fibroblastic neoplasm occurring in the skin of horses, donkeys and mules (Jackson, 1936). It was also used to distinguish it from papilloma, fibroma and fibrosarcoma. Equine sarcoid is a locally aggressive, non-regressing, fibroblastic skin tumour and the most commonly found dermatological skin lesion in equidae (Goodrich *et al*. 1998). A survey among Swiss Warmblood and Freiberger horses found sarcoid tumors among 0.07% and 0.4%, respectively (Dubath M-L 1986). Bovine papillomavirus types 1 and 2 have been detected in sarcoid tumours of horses, donkeys, mules (Lancaster *et al.* 1977; Angelos *et al*. 1991; Bloch *et al*. 1994; Nasir *et al*. 1997; Nasir & Reid 1999; Chambers *et al*. 2003) and more recently in zebras in captivity. (Löhr *et al*. 2005). Sarcoid tumours are associated with BPV but the tumour does not produce infectious virions (Amtmann *et al*. 1980; Lancaster, 1981).

Sarcoid susceptibility in horses was found associated with MHC class I or class II antigens in family studies (Marti *et al*. 1996). In particular, horses with MHC class II haplotypes possessing the ELA-W13 serological specificity had an increased risk for sarcoid tumours (Lazary *et al*. 1985). Associations between class II MHC genes and papillomavirus-induced tumors have also been described in other species. In humans, papilloma virus-induced squamous cell carcinoma of the cervix is more common in women carrying the HLA-DQW3 class II MHC allele (Wank & Thomssen 1991). Apple *et al*. (1994) found a papilloma virus type-specific association between HLA DR-DQ haplotypes in this carcinoma of the cervix. Shope papillomavirus, which induce the development of tumors in the rabbit, are associated with class II MHC genes (Han *et al*. 1992).

The development of a typing system specific to these zebras was created and characterization of the genes using a molecular approach was described in the previous chapter. Single strand conformational polymorphism reproducibly detects genetic variation in DNA and has been used effectively to investigate MHC class II among horses (Fraser, 1998). It has also proved to work well (Chapter 4) to determine the extent of genetic variation for the MHC class II genes of zebras.

The recent increased prevalence of sarcoid tumors among the zebras in Bontebok National Park and Gariep Dam Nature Reserve may be the result of the closed herds becoming inbred for an MHC haplotype that confers increased susceptibility to sarcoid tumors. If this is the case, then one would expect reduced genetic variation of the MHC relative to other parks.

Therefore, the purpose of the study was to determine whether or not the high prevalence of sarcoid among Cape mountain zebra is associated with a MHC haplotype.

5.2 MATERIALS AND METHODS

5.2.1 Study population and sample collection

For comparison of zebras with sarcoid tumors to those without, samples were collected from zebras at game parks in South Africa and testing conducted at the University of Pretoria (UP). To implement the typing system, blood samples were collected in EDTA-buffered tubes by venipuncture of the jugular vein and stored at 4 °C from a total of 34 zebras located in different national parks in South Africa. Blood were collected from affected Cape mountain zebra (*Equus zebra zebra*) in the Gariep Dam Nature Reserve (Free State Province) (n=18), Bontebok National Park (Western Cape Province) (n=4) and Mountain Zebra National Park (n=2). Blood were collected from zebras in parks where no sarcoid tumours have been observed before, from

Hartmann's mountain zebra (*Equus zebra hartmannae*) (n=3) in the Augrabies National Park (Northern Cape Province) and the Cederberg Wilderness Area (Western Cape Province) (n=2); Burchell's zebra (*Equus quagga burchelli*) (n=2) in the Karoo National Park (Western Cape Province) and Cape mountain zebra in the Karoo National Park (n=3). The age of the animals varied between 3-15 years. These samples were processed and the typing system applied in the Molecular Laboratory, Department of Veterinary Tropical Diseases, UP, Onderstepoort, South Africa.

5.2.2 DNA extraction

DNA was extracted from 200 µl of blood collected from the above mentioned zebras using the QIAamp®DNA extraction kit (Southern Cross Biotechnologies) according to the manufacturer's instructions. Extracted DNA was eluted in 100 µl elution buffer and stored at 4 °C until further analysis.

5.2.3 Conventional PCR amplification of the second exon class II

Class II DRB, and DQB genes were PCR amplified using the primers and conditions as described in Chapter 4. The amplified regions lie within the second exon of both genes and amplicons were used for SSCP typing.

5.2.4 Molecular cloning and sequencing

The primer pairs for MHC class II DQB and DBR that were used to amplify the genomic sequences were described in **Table 5** (Chapter 4). The amplicons were purified, cloned into the pGEM®-T vector (Promega pGEM-T Easy Vector System, Promega, Madison, USA), and transformed into competent JM 109 *E. coli* cells (Promega, Madison, USA). Recombinant plasmid DNA was isolated, directly sequenced using the ABI BigDye™ Terminator Cycle Sequencing Ready Reaction KIT (PE Applied Biosystems) and analysed on an ABI 3100 sequencer. Sequencing data were assembled and edited with the GAP4 program of the Staden package (version 1.6.0 for Windows) and aligned with published sequences of related genera using ClustalX (version 1.81 for Windows). A BLAST search was performed using the Blastn algorithm to confirm the identity of the DNA fragments as DRB and DQB products.

5.2.5 MHC Typing: Single-Strand Conformational Polymorphism Analysis

Analysis was based on the SSCP method originally described by Orita *et al*. (1989). The SSCP gels consisted of a 10% w/v acrylamide:bis-acrylamide (37.5:1) (BioRad, South Africa) 0.5X TBE buffer (44.5mM boric acid, 1mM Na_2-EDTA, pH=8.4) (Qiagen, Southern Cross Biotechnology, South Africa), 0.093% w/v ammonium persulphate (MERCK, South Africa) and 0.08% v/v N,N,N',N'-tetramethylethylenediamine (TEMED) (MERCK, South Africa). The samples consisted of PCR products from genomic DNA as well as cloned PCR products.

Samples (8 µl water + 3 µl loading dye + 2 µl PCR products) were denatured in a heating block at 90 °C for 10 minutes and put immediately on ice until loaded. The gels were electrophoresed in 0.5X TBE buffer on a Protean II xi cell gel apparatus (BioRad Laboratories Inc., Melville, NY) at 100 volts for 20 hours at 20 °C. The SSCP gel was visualized by staining for 10 minutes in a 0.5X TBE with 1 ul of a 1 mg/ml stock solution of ethidium bromide, and photographed under UV trans-illumination. The gels were scored by counting the number of fragments visible on the gel.

5.3 RESULTS

5.3.1 Amplification of MHC DNA fragments

Amplifications of Cape mountain zebra DNA using primers outlined in **Table 5** (Chapter 4) were successful for DQB and DRB and produced DNA fragments the same size (210 and 276 bp) in Cape mountain zebras (*Ezz*) as in horses and Hartmann's zebras (*Ezh*).

5.3.2 Comparison of SSCP DRB and DQB profiles for zebras with and without sarcoid tumours

5.3.2.1 DRB

None of the bands on the gels occurred exclusively among zebras with sarcoid tumours or zebras without sarcoid tumours.

Among the profiles of the 13 zebras illustrated in **Figure 19** there were eight different phenotypes, five among the zebras with sarcoid tumours and three among the zebras without sarcoid tumours. Of the 11 bands scored on the gel, eight were polymorphic. No association occurred exclusively among the zebras with sarcoid tumours or those without sarcoid tumours.

5.3.2.2 DQB

As in DRB none of the bands on the gels occurred exclusively among zebras with sarcoid tumours or zebras without sarcoid tumours. Among the profiles of the eight zebras illustrated in **Figure 20** there were seven different phenotypes, five among the zebras with sarcoid tumours and two among the zebras without sarcoid tumours. Of the 12 bands scored on the gel, 10 were polymorphic. No association occurred exclusively among the zebras with sarcoid tumours or those without.

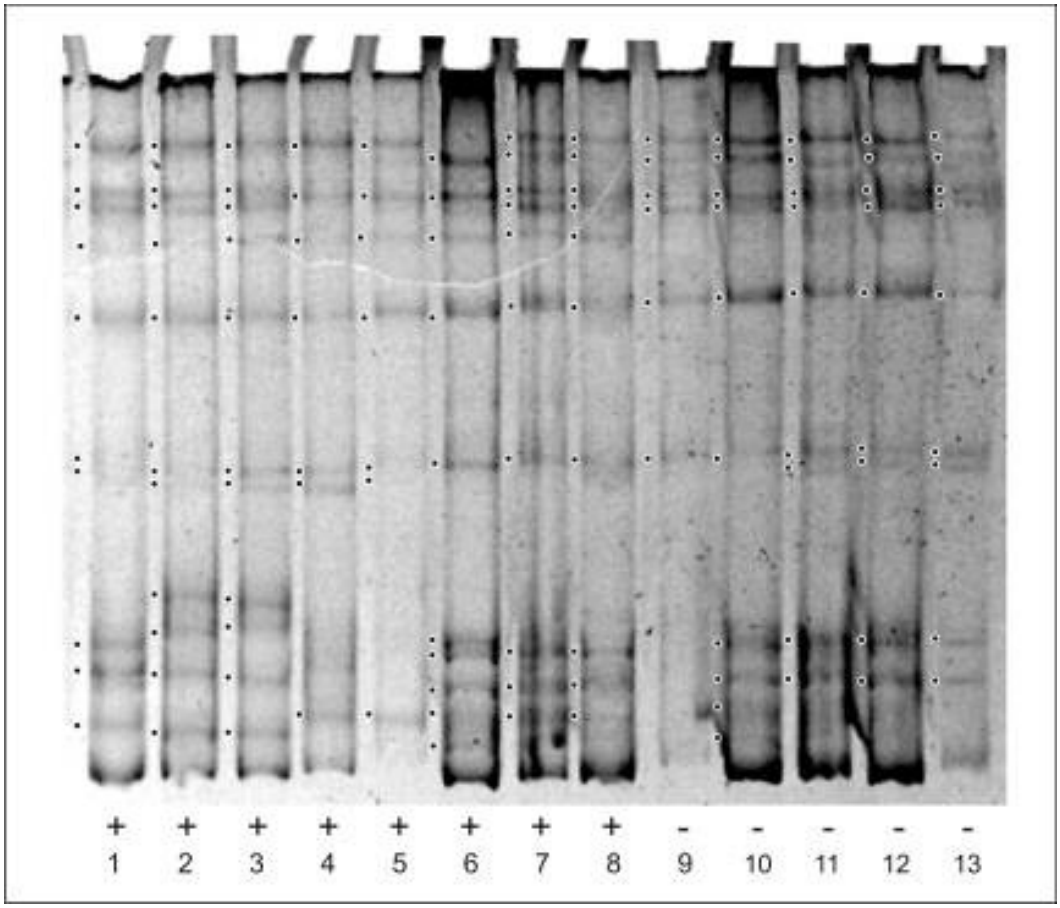


Figure 19 DRB SSCP patterns from genomic DNA amplifications for different zebra demonstrated in reverse contrast to the ethidium bromide stained gel: Lane 1-8 represent *Equus zebra zebra* (*Ezz*) numbers 1, 5, 7, 32, 35, 79, 82, 83, all sarcoid positive, Lane 9-13: *Ezz* 102, *Equus zebra hartmannae* (*Ezh*) 130, *Equus quagga burchelli* (*Eqb*) 158, *Eqb*160, *Ezh* 176, all sarcoid negative.

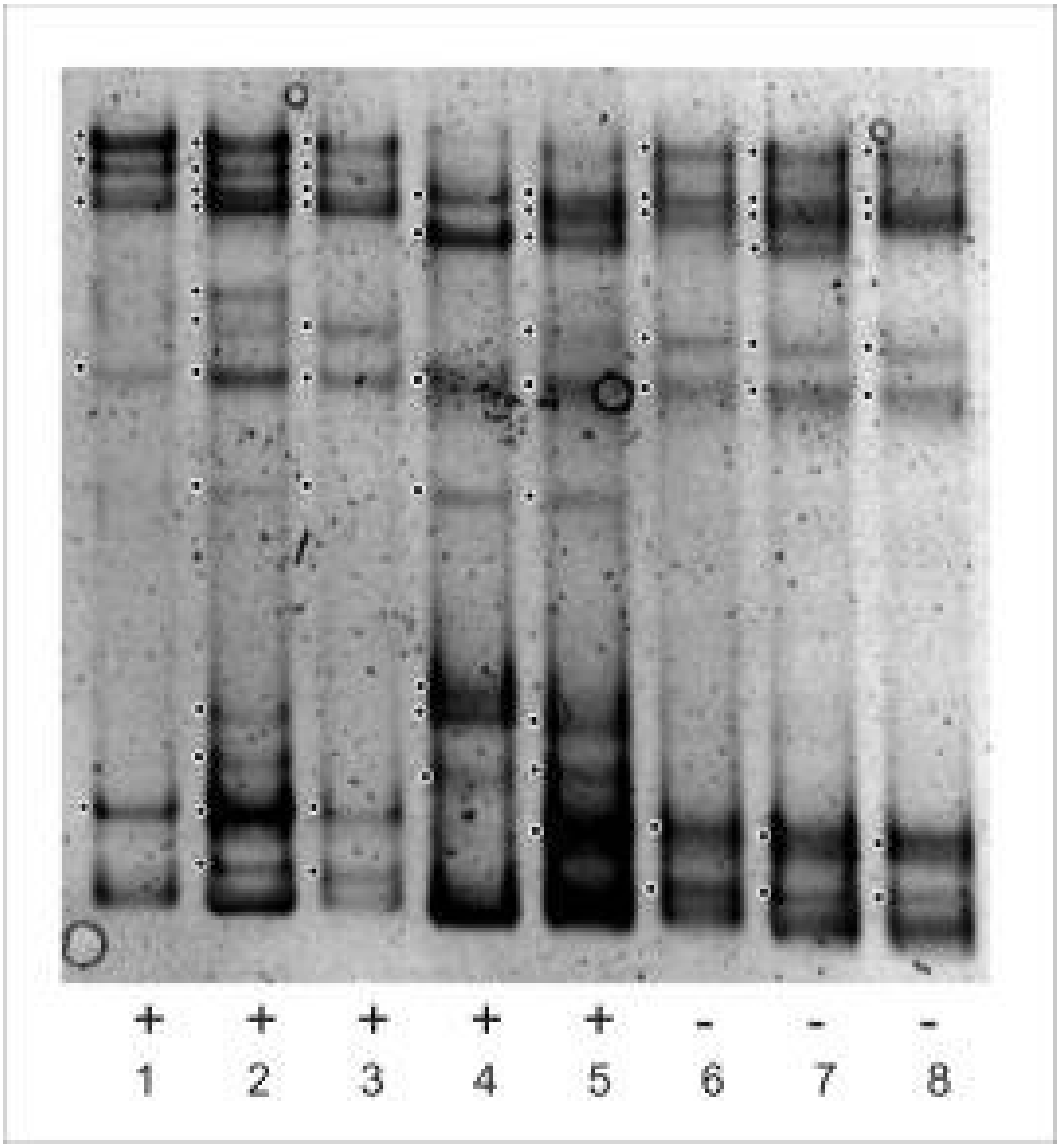
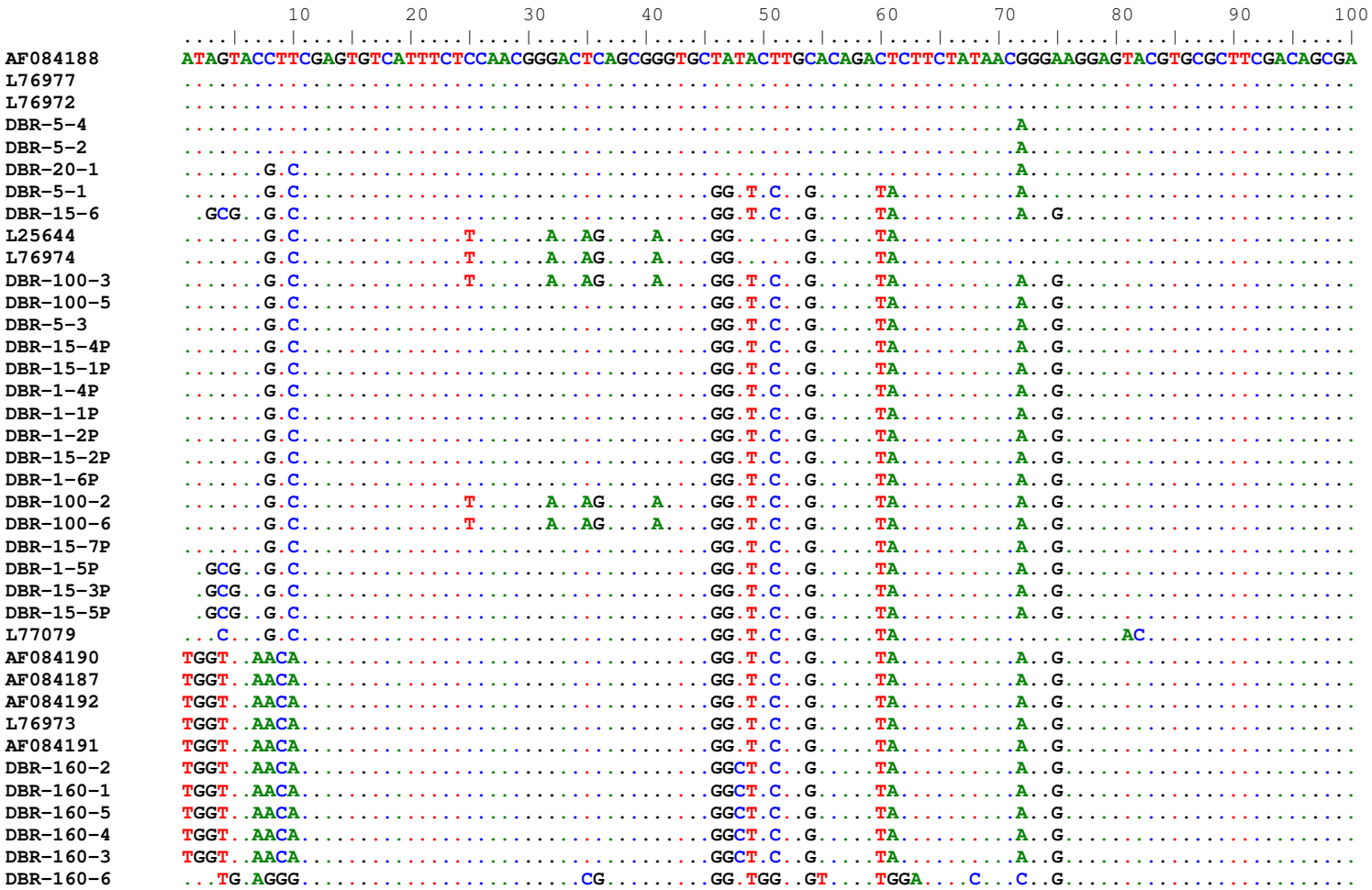
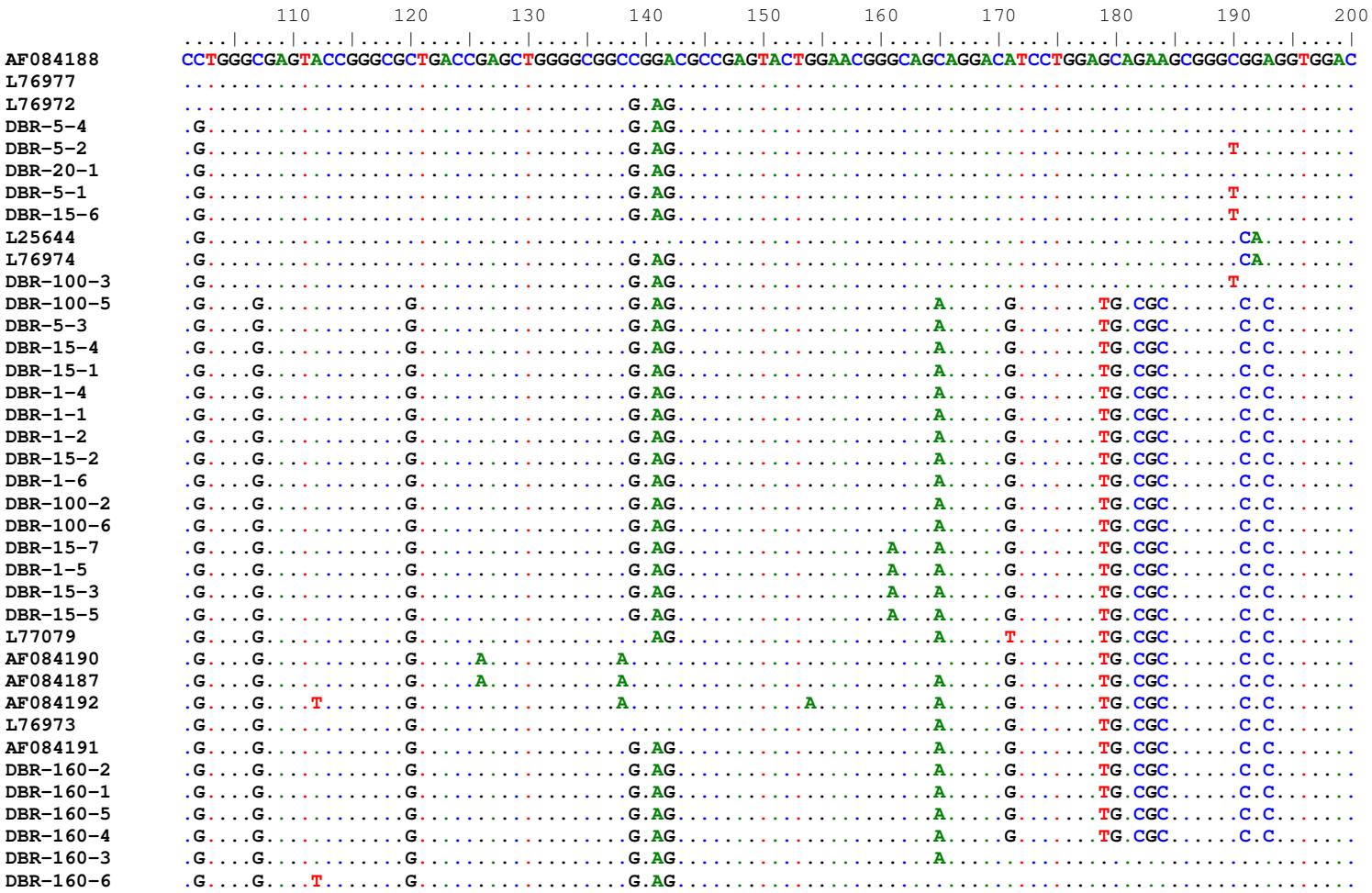


Figure 20 DQB SSCP patterns from genomic DNA amplifications for zebra with and without sarcoid demonstrated in reverse contrast to the ethidium bromide stained gel: Lane 1-5 sarcoid positive zebra *Ezz* numbers 1, 7, 79, 91, 97. Lanes 6-8 sarcoid negative zebra *Ezz* numbers 100, 109, 112.

5.3.3 Sequencing

DRB second exon sequences for clones from sarcoid affected zebra (*Ezz* 1, 5, 15, 20) and sarcoid unaffected zebra *Ezz* 100 and *Equus zebra burchelli* (*Ezb*) 160 are tabulated in **Figure 21** compared to equine DRB sequences obtained from GenBank. Sequences from 17 clones tested from four sarcoid affected zebras and 10 clones tested from two healthy zebras showed genetic diversion among sarcoid-affected as well as healthy zebras. BLAST results showed high similarity with *Equus przewalski* class II antigen DBR gene accession numbers (AF084177, AF084178, AF084190, AF08491, AF08492).


10 20 30 40 50 60 70 80 90 100
AF084188 ATAGTACCTTCGAGTGTCATTTCTCCAACGGGACTCAGCGGGTGCTATACTTGCACAGACTCTTCTATAACGGGAAGGAGTACGTGCGCTTCGACAGCGA
L76977
L76972
DBR-5-4
DBR-5-2
DBR-20-1
DBR-5-1
DBR-15-6
L25644
L76974
DBR-100-3
DBR-100-5
DBR-5-3
DBR-15-4P
DBR-15-1P
DBR-1-4P
DBR-1-1P
DBR-1-2P
DBR-15-2P
DBR-1-6P
DBR-100-2
DBR-100-6
DBR-15-7P
DBR-1-5P
DBR-15-3P
DBR-15-5P
L77079
AF084190
AF084187
AF084192
L76973
AF084191
DBR-160-2
DBR-160-1
DBR-160-5
DBR-160-4
DBR-160-3
DBR-160-6


110 120 130 140 150 160 170 180 190 200
AF084188 CCTGGGCGAGTACCGGGCGCTGACCGAGCTGGGGCGGCCGGACGCCGAGTACTGGAACGGGCAGCAGGACATCCTGGAGCAGAAGCGGGCGGAGGTGGAC
L76977
L76972
DBR-5-4
DBR-5-2
DBR-20-1
DBR-5-1
DBR-15-6
L25644
L76974
DBR-100-3
DBR-100-5
DBR-5-3
DBR-15-4
DBR-15-1
DBR-1-4
DBR-1-1
DBR-1-2
DBR-15-2
DBR-1-6
DBR-100-2
DBR-100-6
DBR-15-7
DBR-1-5
DBR-15-3
DBR-15-5
L77079
AF084190
AF084187
AF084192
L76973
AF084191
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DBR-160-5
DBR-160-4
DBR-160-3
DBR-160-6

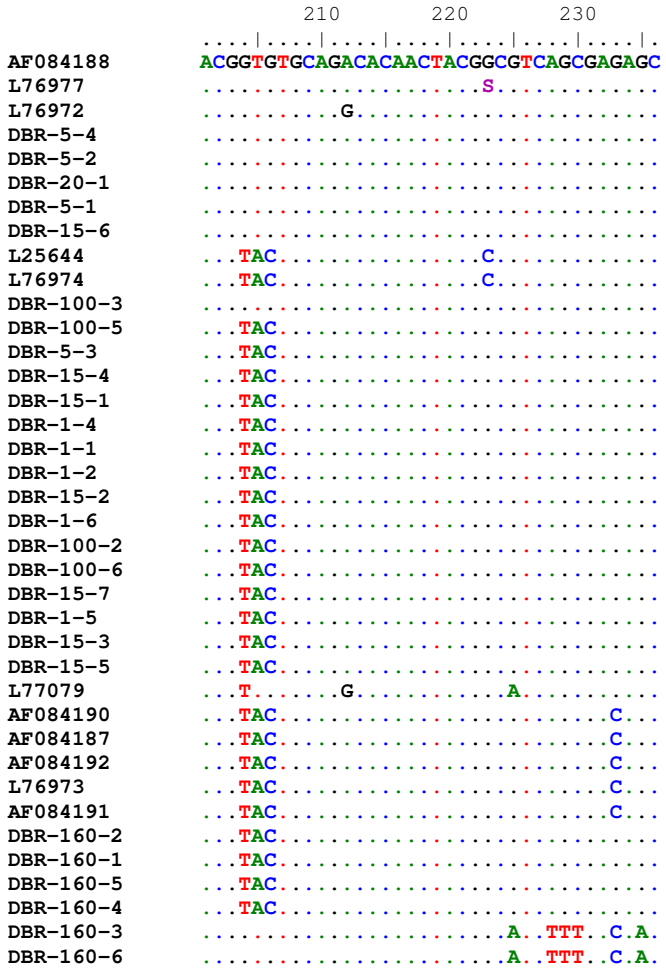
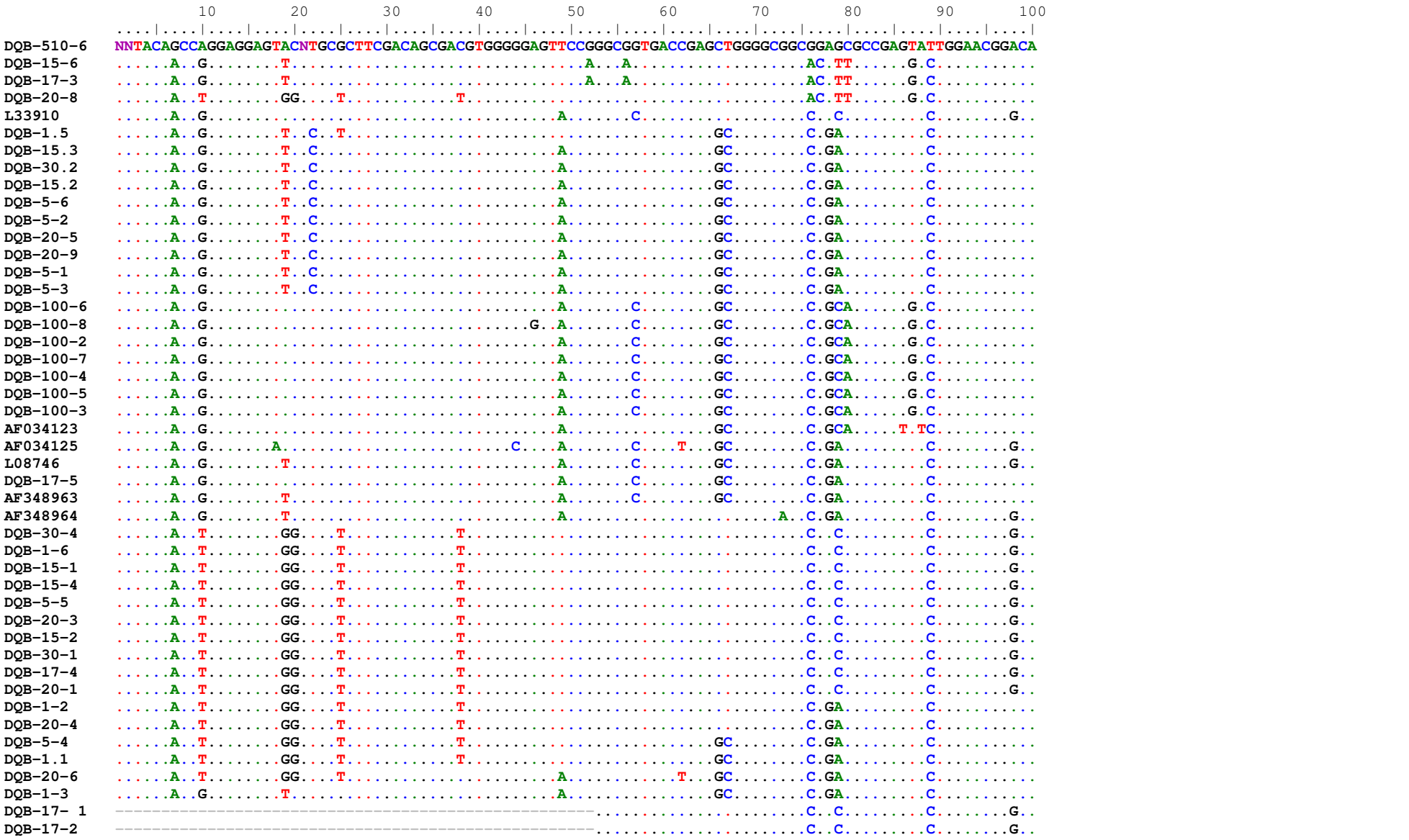


Figure 21 Alignment of nucleotide sequences for the 2nd exon of MHC class II DBR clones (designated "DRB-animal number-clone number") from *Ezz* 1, 5, 15, 20, 100, and *Ezh* 160. (Ezz 1, 5, 15, 20 are sarcoid positive) *Eqpz* accession numbers AF084187; AF084188; AF084190; AF184191; AF084192; and *Ec* accession numbers L25644; L76972; L76973; L76974; L76977; L77079 are used as references.


10 20 30 40 50 60 70 80 90 100
DQB-510-6 NNTACAGCCAGGAGGAGTACNTGCGCTTCGACAGCGACGTGGGGGAGTTCCGGGCGGTGACCGAGCTGGGGCGGCGGAGCGCCGAGTATTGGAACGGACA
DQB-15-6
DQB-17-3
DQB-20-8
L33910
DQB-1.5
DQB-15.3
DQB-30.2
DQB-15.2
DQB-5-6
DQB-5-2
DQB-20-5
DQB-20-9
DQB-5-1
DQB-5-3
DQB-100-6
DQB-100-8
DQB-100-2
DQB-100-7
DQB-100-4
DQB-100-5
DQB-100-3
AF034123
AF034125
L08746
DQB-17-5
AF348963
AF348964
DQB-30-4
DQB-1-6
DQB-15-1
DQB-15-4
DQB-5-5
DQB-20-3
DQB-15-2
DQB-30-1
DQB-17-4
DQB-20-1
DQB-1-2
DQB-20-4
DQB-5-4
DQB-1.1
DQB-20-6
DQB-1-3
DQB-17- 1
DQB-17-2

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                  110       120
          ....|....|....|....|....|..
DQB-510-6 GAAGGACATCCTGGAGGAGTACCGGGC
DQB-15-6  .......G........C..A.G.....
DQB-17-3  .......G........C..A.G.....
DQB-20-8  .......G........C..A.G.....
L33910    .......G........C..ACG.....
DQB-1-5   ..............AG.ACG.....
DQB-15-3  ..............AG.ACG.....
DQB-30-2  ..............AG.ACG.....
DQB-15-2  ..............AG.ACG.....
DQB-5-6   ..............AG.ACG.....
DQB-5-2   ..............AG.ACG.....
DQB-20-5  ..............AG.ACG.....
DQB-20-9  ..............AG.ACG.....
DQB-5-1   ..............AG.ACG.....
DQB-5-3   ..............AG.ACG.....
DQB-100-6 ..............AG.ACGT....
DQB-100-8 ..............AG.ACGT....
DQB-100-2 ..............AG.ACGT....
DQB-100-7 ..............AG.ACGT....
DQB-100-4 ..............AG.ACGT....
DQB-100-5 ..............AG.ACGT....
DQB-100-3 ..............AG.ACGT....
AF034123  ..............CAG.ACGT....
AF034125  ..............C..ACG.....
L08746    .......T........C..ACG.....
DQB-17-5  ..............A..ACG.....
AF348963  ..............CG.ACG.....
AF348964  ..............A..ACGT....
DQB-30-4  ..............CG.GTG.....
DQB-1-6   ..............CG.GTG.....
DQB-15.1  ..............CG.GTG.....
DQB-15.4  ..............CG.GTG.....
DQB-5-5   ..............CG.GTG.....
DQB-20-3  ..............CG.GTG.....
DQB-15-2  ..............CG.GTG.....
DQB-30-1  ..............CG.GTG.....
DQB-17-4  ..............CG.GTG.....
DQB-20-1  ..............AG.ACG.....
DQB-1-2   ..............AG.ACG.....
DQB-20-4  ..............AG.ACG.....
DQB-5-4   ..............AG.ACG.....
DQB-1-1   ..............AG.ACG.....
DQB-20-6  ..............AG.ACG.....
DQB-1-3   ..............AG.ACG.....
DQB-17-1  ..............CG.GTG.....
DQB-17-2  ..............CG.GTG.....
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Figure 22 Alignment of nucleotide sequences for the 2nd exon of MHC class II DQB clones (designated "DQB-animal number-clone number") from *Ezz* 1, 5, 15, 20, 30, (sarcoid positive) and *Ezz* 17, 100 and *Ezh* 510 (sarcoid negative). *Ec* accession numbers L33910; L08746; AF348963; AF348964; *Ea* accession numbers AF034123; AF034125 are used as references.

DQB second exon sequences for clones from sarcoid affected zebra (*Ezz* 1, 5, 15, 20, 30) and sarcoid unaffected zebra *Ezz* 17 and 100 are tabulated in **Figure 22** compared to equine sequences obtained from GenBank to identify the DNA fragments as DQB products. Sequences from 26 clones from seven sarcoid affected zebras and 10 clones from two healthy zebras showed genetic diversion among sarcoid as well as healthy zebras. BLAST results showed a high similarity (~93%) with MHC class II antigen (ELA-DQB) gene, *Ea* reference number (U31775) and *Ec* reference number (AF348963).

5.4 DISCUSSION

The system developed for typing MHC in Hartmann's zebras by using SSCP worked well for the Cape mountain zebras. PCR primers developed for amplification of the horse MHC genes also worked well for amplifying the Cape mountain zebras MHC gene. DNA sequencing confirmed the identity of the DNA fragments as being MHC class II gene products and confirmed that the DNA fragments under investigation were for MHC-DQB and MHC-DRB based on homology to the DNA sequence of comparable horse genes.

The hypothesis that the herd was inbred and the increase of sarcoid tumours was due to loss of variation in the MHC region could not be confirmed. However, the number of DNA fragments seen among the different groups of zebras in South Africa was similar to that observed for zoo maintained zebras from the United States and comparable to that seen for domestic horses. Different electrophoresis apparatuses were used in Kentucky, where the typing system was developed, and South Africa, where the sarcoid affected herds were investigated. The unit used in Kentucky was longer, allowing slightly more resolution.

Whilst recombination is thought to be reduced in MHC of many species, it could still occur, and in the absence of the identification of the causative mutation it could be that the markers used will show different frequencies rather than categorical differences. The allele frequencies of the two populations could have been statistically compared to see if any of the MHC alleles are significantly compared with sarcoid development. The age range of the zebras (3-12 years) might however change the allele distribution, as negative animals might develop sarcoids in the future. The sample size used is too small for this type of experiment, and it is rare for susceptible loci that one allele is observed exclusively in the sarcoid-affected or control population.

Despite the lower resolution for SSCP typing, variation was readily found for both DRB and DQB among the zebras affected with sarcoid tumours. Furthermore, there were no fragments found which were unique to either the affected or unaffected zebras. These results do not support a genetic influence of the MHC for the very high occurrence of sarcoid tumors among zebra in these parks and the hypothesis that the zebras become inbred for the MHC, leading to homozygosity for a susceptible haplotype, clearly did not occur. It does not exclude the fact that the MHC can be involved, there could be a haplotype that has become more frequent and that SSCP, being an insensitive test, did not discern it from others. The cause for the high appearance of sarcoid in these parks should also be investigated elsewhere, perhaps by studies of environmental factors causing immunosuppression and thereby stimulating growth of sarcoid tumours among zebras.

5.5 REFERENCES

Amtmann E, Muller H, Sauer G, 1980. Equine connective tissue tumours contain unintegrated bovine papillomavirus DNA. *Journal of Virology* 35:962-964.

Angelos JA, Marti E, Lazary S, Carmichael LE, 1991. Characterization of BPV-like DNA in equine sarcoids. *Archives of Virology*. 119: 95-109.

Apple RJ, Ehrlich HA, Klitz W, Manos MM, Becker TM, Wheeler CM, 1994. HLA DR-DQ associations with cervical carcinoma show papillomavirus-type specificity. *Nature Genetics* 6:157-162.

Bloch N, Breen M, Spradbrow PB, 1994. Genomic sequences of bovine papillomaviruses in formalin-fixed sarcoids from Australian horses revealed by polymerase chain reaction. *Veterinary Microbiology* 41:163-172.

Chambers G, Ellsmore VA, O'Brien PM, Reid SWJ, Love S, Campo MS, Nasir L, 2003. Sequence variants of bovine papillomavirus E5 detected in equine sarcoids. *Virus Research* 96:141-145.

Dubath M-L, 1986. Recherche d'association entre le systeme ELA et une predisposition aus sarcoides equines, *PhD thesis*. Berne, Switzerland, Faculty of Veterinary Medicine, University of Berne. 55p.

Fraser DG, 1998. Studies on the organization and the polymorphism of equine MHC Class II genes. *PhD Thesis*, University of Kentucky, Lexington, Kentucky. 115p.

Goodrich L, Gerber H, Marti E, Antczak DF, 1998. Equine sarcoids. *Veterinary Clinics of North America-Equine Practice*. 14: 607-623.

Han R, Breitburd F, Marche PN, Orth G, 1992. Linkage of regression and malignant conversion of rabbit viral papillomas to MHC class II genes. *Nature* 356:66-68.

International Union for the Conservation of Nature and Natural Resources (*IUCN*), 2009. The IUCN Red List of Threatened Species™ (version 2009.1) [cited August 2009]. Available from: (http://www.iucnredlist.org).

Jackson C, 1936. The incidence and pathology of tumours of domesticated animals in South Africa: A study of the Onderstepoort collection of neoplasms with special reference to their histopathology. *Onderstepoort Journal of Veterinary Science and Animal Industry* 6:1-460.

Lancaster WD, 1981. Apparent lack of integration of bovine papilloma virus DNA in virus induced equine and bovine tumor cells and virus-transformed mouse cells. *Virology*. 108:251-255.

Lancaster WD, Olson C, Meinke W, 1977. Bovine papilloma virus: presence of virus-specific DNA sequences in naturally occurring equine tumors. *Proceedings of the National Academy of Sciences of the United States of America* 74:524-528.

Lange AL, 2004. Ongoing investigation into an outbreak of sarcoid tumours in the Cape mountain zebra of the Bontebok National Park, Swellendam, *A report presented to SANParks*.

Lazary S, Gerber H, Glatt PA, Straub R, 1985. Equine leucocyte antigens in sarcoid affected horses. *Equine Veterinary Journal* 17:283-286.

Löhr CV, Juan-Sallés C, Rosas-Rosas A, Parás García A, Garner MM, Teifke JP, 2005. Sarcoids in captive zebras (*Equus burchellii*): association with bovine papillomavirus type 1 infection. *Journal of Zoo Wildlife Medicine* 36:74-81.

Marti E, Szalai G, Antczak AF, Bailey E, Gerber H, Lazary S, 1996. The Equine Major Histocompatibility Complex. In: *The Major Histocompatibility Complex Region of Domestic Animal Species*. Shook LB, Lamont SJ, (eds), CRC Press, Inc., Boca Raton, Florida. 245-267.

Nasir L, McFarlane ST, Torrontegui BO, Reid SWJ, 1997. Screening for bovine papilloma virus in the peripheral cells of donkeys with and without sarcoids. *Research in Veterinary Science*. 63:289-290.

Nasir L, Reid SWJ, 1999. Bovine papillomaviral gene expression in equine sarcoid tumours. *Virus Research* 61:171-175.

Nel PJ, Bertschinger H, Williams J, Thompson PN, 2006. 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* 77:184-190.

Novellie P, Lindeque M, Lindeque P, Lloyd PH, Koen J, 2002. Status and Action Plan for the Mountain Zebra (*Equus zebra*). In: *Equids: zebras, asses and horses: status survey and conservation action plan*. Moehlman PD, (ed), IUCN / SSC, Gland, Switzerland.

Orita M, Iwahana H, Kanazawa H, Hayashi K, Sekija T, 1989. Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. *Proceedings of the National Academy of Sciences of the United States of America* 86:2766-2770.

Penzhorn BL, 2003. Social organization of the Cape mountain zebra *Equus zebra zebra* in the Mountain Zebra National Park. *Koedoe* 22:115-156.

Penzhorn BL, Novellie PA, 1991. Some behavioural traits of Cape mountain zebras (*Equus zebra zebra*) and their implications for the management of a small conservation area. *Applied Animal Behavior Science* 29:293-299.

Wank R, Thomsson C, 1991. High risk of squamous cell carcinoma of the cervix for women with HLA-DQw3. *Nature* (London). 352:723-725.