

## CHAPTER SEVEN: CONCLUSION

The occurrence of sarcoid in the endangered Cape mountain zebras in the Bontebok National Park and Gariiep Dam Nature Reserve in South Africa is a reality which needs urgent attention. A very high incidence of this condition was reported (24% and 53%) of the populations of this vulnerable species. The zebras were severely stressed and were unsightly for the many tourists visiting these parks. Their condition deteriorated as result of the growths and they become debilitated and died. The study was performed to investigate if bovine papillomavirus (BPV) is associated with sarcoids in the free-roaming zebras, to develop a real-time polymerase chain reaction (PCR) diagnostic method to detect and distinguish between BPV-1 and -2, to study the phylogenetic relationships isolated from sarcoids in the zebras in comparison with other equidae species and to determine whether or not the high prevalence of sarcoids in these herds is associated with a major histocompatibility complex (MHC) haplotype. Furthermore, to determine ultrastructurally if BPV is present in the papillomas in the skin of the giraffe and a sable antelope, and if so, to detect and distinguish between BPV-1 and -2 using the real-time PCR method.

Although BPV-1 and -2 are associated with sarcoids in horses, donkeys, and mules as well as in captive zebras their presence in free-roaming zebras has not been previously described. Cattle, which might be a source of the virus, are not associated with the parks and, although neighbouring farms might previously have harboured cattle, both of the affected parks are not situated in cattle farming districts and it is highly unlikely that large herds of cattle ever previously occurred in the regions concerned. The Bontebok National Park, however, did incorporate an area which was earlier used as a racehorse-track, where from time to time horses were grouped together. The Bontebok National Park was proclaimed in 1931 and the Gariiep Dam Nature reserve in 1972. Since then zebras have been free-roaming and there has been no human intervention.

Histologically, the lesions in the zebras exhibited the identical features to those of sarcoids in the horse but, as in the horse, no intact virus particles could be demonstrated electron microscopically. However BPV DNA was demonstrated by PCR and RFLP. It was shown that both BPV-1 and BPV-2 DNA are indeed present in sarcoids in these zebra. Single BPV-1 or BPV-2 infections as well as mixed infections occurred in individual animals. The virus DNA was only detected from

sarcoid tissue and was not found in the blood or unaffected skin of the animals when examined by conventional PCR. The reason why the autogenous vaccine used to combat the disease had a higher therapeutic rate in the one park could be explained as it was shown that the vaccine used there contained both BPV-1 and -2 whereas in the other park, the vaccine used only contained BPV-1 and several cases did not respond to treatment.

A 637 bp product was cloned and sequenced to prove that the product is indeed bovine papillomavirus in which, in a broad selection of papillomaviruses compiled in a phylogenetic tree, the phylogenetic position of BPV-1 and -2 is well nested within the delta-epsilon-zeta papillomavirus superclade which includes papillomaviruses infecting several different hosts. This confirms the theory that BPV plays an important role in the aetiology and pathogenesis of equine (which includes those of the zebras in this investigation) sarcoids.

When the evolutionary relationships of the E5 gene sequences obtained from zebra BPV were determined the zebra sequences clustered not only with BPV-1 but also with BPV-2 as sister clades to other related equid sequences. The age for the most recent common ancestor for BPV-1 variants was estimated to be 1.4 Mya, while that for BPV-2 variants was estimated to be 0.55 Mya. The age for the most recent common ancestor for BPV-1 and -2 has been estimated to be 5.34 Mya. This can be used in further studies to explain the virus-host switch and adaptations to the new host. Further studies should, however, rather include sequences of the more variable upstream regulatory region, which is positioned between the L1 and L6 genes, where more BPV variants have been described.

A rapid, sensitive and reliable real-time PCR assay to detect and distinguish between BPV-1 and -2 infections in the zebras was developed. Using this sensitive method it was found that the virus DNA is also present in the normal skin and in the blood of healthy animals even from parks where sarcoids do not occur. The concentration of the virus found varied, being the highest in the sarcoid tissue, followed by the skin and lastly the blood, and was evident by the different threshold values of the amplification curves.

The virus is therefore present in healthy animals. The infection is however, latent and the animals show no clinical signs of disease. The immune system of an infected animal plays an important role in the outcome of papillomavirus infections as immune suppression and physical trauma can reactivate latent asymptomatic papillomavirus infections and clinical signs appear. A systematic

investigation of the immune response in animals suffering from sarcoid should yield results of great interest.

The reason why certain animals succumb to the disease was investigated by investigating their genetic background. The small populations all originated from the Mountain Zebra National Park where the zebras are protected and are progeny of the original 11 individual animals. It has been suggested that in the horse a hereditary predisposition exists, which was established by the strong association between prevalence of sarcoid and genes in or near the equine MHC. The precise cause for the association of MHC class II in horses with sarcoid tumours, however, has never been determined. The ELA DW13 serological specificity, associated with some sarcoid cases, is simply a genetic marker for a haplotype with multiple loci of class II genes.

A typing system to investigate the MHC haplotypes in the zebra was developed using SSCP. No differences in phenotypes were observed between the zebras in the affected parks based on the presence of sarcoid tumours. The zebras in these parks, although inbred herds, had a variation at the MHC for class II (DRB and DQB) genes which give them enough genetic diversity and protection against a variety of pathogens. The increased occurrence of sarcoid tumours in the zebras in Bontebok National Park and Gariep Dam Nature Reserve is not due to inbreeding and homozygosity at the MHC since the zebras do show variation. From the results obtained in this study no genetic marker could be identified for sarcoids in the zebra. With new analytical methods, for instance, single nucleotide polymorphism, a causative mutation might be found, where regions of the genome with and without disease are compared.

The two parks in which the affected zebras occur are the smallest parks in which Cape mountain zebras are present. The habitat in both parks is not ideal for the species. In Bontebok National Park there is a general conflict of interest: that of managing the herbivores *versus* that of flora conservation. Two red-listed herbivore species the bontebok (*Damaliscus pygarrus dorcas*) and the Cape mountain zebra (*Equus zebra zebra*) are protected here. The red hartebeest (*Alcelaphus buselaphus*) also occurs here. The suitability of the habitat is ideal for bontebok conservation but is only marginal for zebras. The vegetation consists mainly of fynbos and renosterveld. The fynbos biome includes both fynbos and renosterveld, in which the shrubs have small hard leaves but there are very few trees and grasses. In renosterveld, a shrub known as climber's friend or "steekbossie" (*Cliffortia ruscifolia*) occurs. This is a prickly bush with very sharp leaves which can easily traumatise the skin. Renosterbos (*Elytropappus rhinocerotis*) is characteristically dominant and is very unpalatable. All the shrubs in the renosterveld are characterized by possessing small, tough,

grey leaves. In the past, grasses were abundant in renosterveld but this led to their being overgrazed and replacement by shrubs; therefore grasses are uncommon today.

The zebras in Gariiep Dam Nature Reserve also compete with other herbivores for the available grazing, which is classified as eastern mixed Nama Karoo. The vegetation consists of grassy dwarf shrubs interspersed with tassel bristlegrass (*Aristida congesta*) and Lehmann's lovegrass (*Eriocephalus ericoides*). The seeds of both these grass species possess very sharp awns which could cause superficial wounds to the skin. This reserve is situated outside the normal habitat of the Cape mountain zebra which favour mountainous areas containing their preferred type of grazing.

Tick infestations on the zebras in both parks also occur. The ticks traumatise the skin, especially its ventral aspects. It is felt that their role as possible vectors in the development of the disease should also be investigated to determine if the papillomavirus is transgenic in the ticks and what role they could have in the host-switch of the papillomavirus. The possible role of other vectors, such as flies, particularly, those which bite, and oxpeckers (*Buphagus* species) could play a role and their importance should also be investigated. They could play a role in virus host switch and virus evolution upon host switching.

South Africa is at times subjected, both generally and regionally, to severe droughts which could aggravate some of the the above conditions leading to stress in the animals and hence immunosuppression.

In two other species of wildlife, a giraffe and a sable antelope, papillomavirus was detected electron microscopically in cutaneous fibropapillomas. Histopathologically, the lesions in both species of animal showed features similar to those of equine sarcoid, but those in the giraffe showed histopathological evidence of malignant fibroblast pleomorphism. Since one of the giraffes tested in this study did not reveal any evidence of the presence of virus particles histologically, immunohistochemically or ultra structurally, it is considered that it was the end-point of BPV infection. Infection with the virus was, however, still detected by real-time PCR which is capable of detecting 1.5 gene copies of the virus. Clinical manifestation of a latent infection with papillomavirus in these animals may have been provoked by stress. In the case of the sable antelope, this could have been due to the fact that it was being kept in a habitat to which it was not accustomed and which could have been exacerbated by transport and resultant immunosuppression. In the case of the giraffes, one can speculate that drought conditions and the resultant high tannin content of the *Acacia*-trees, as a result of overbrowsing, as the latter are their preferred diet, could

act as a co-factor in BPV-associated carcinogenesis as occurs in cattle following bracken fern (*Pteridium aquilinum*) ingestion. This hypothesis requires investigation. No co-factor has as yet been identified for BPV-associated fibropapillomas or sarcoids.

In summary this was the first study demonstrating the presence of BPV-1 and -2 in sarcoid tissue of free-roaming zebra as well as the informative finding that BPV also occur in the skin and blood of clinical healthy animals, even in areas where sarcoid has never been observed. From this study, it is clear that inbreeding leading to homozygosity for a sarcoid susceptible haplotype is not the cause of sarcoid development although it does not exclude the possibility that the MHC is not involved. Animals succumb to the disease when they become immune suppressed and the virus overcome the immune response of the host. Game park officials should recognize stress-causing factors in game and act accordingly to prevent or minimize the disease.