



# **DETECTION AND CHARACTERIZATION OF PAPILLOMAVIRUS IN ZEBRA (*Equus zebra*) AND OTHER SOUTH AFRICAN WILDLIFE SPECIES**

By

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Dedicated to my sons Wim and Gerhard

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# SUMMARY

## DETECTION AND CHARACTERIZATION OF PAPILLOMAVIRUS IN ZEBRA (*Equus zebra*) AND OTHER SOUTH AFRICAN WILDLIFE SPECIES

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### For the **PhD** degree

Sarcoid-like tumours have been reported in Cape mountain zebra (*Equus zebra zebra*) in two South African game parks recently. These tumours caused severe distress to the animals and also made them unsightly for tourists visiting the parks.

The aim of this investigation was to identify and characterize the infectious agent considered to be involved in the aetiology of sarcoid in the Cape mountain zebra. Bovine papillomaviruses (BPV) -1 and -2 deoxyribonucleic acid (DNA) were detected by the polymerase chain reaction (PCR) in sarcoid tumour tissue, but not from blood specimens or unaffected skin. Differentiation between BPV-1 and -2 was made by using the restriction endonuclease *Bst*XI on PCR products of the E5 open reading frame (ORF).

A hybridization probe real-time assay was developed for the specific and sensitive detection and differentiation of BPV-1 and -2 DNA in blood, skin and sarcoid tumour samples. For the specific detection of BPV-1, an increase in fluorescence was detected at 640 nm and of BPV-2 at 705 nm. The test is extremely sensitive and able to detect 1.5 genome copies/reaction.

The presence of BPV-1 and -2 DNA could be demonstrated in the blood of sarcoid-affected and -unaffected zebras even in the blood of zebras from parks where sarcoids have never been observed.

The phylogenetic relationships of the papillomaviruses detected in tumours in the Cape mountain zebra in comparison with a broad selection of papillomavirus sequences available in GenBank were compiled. The papillomavirus sequences retrieved from the zebras were identified as variants of either BPV-1 or BPV-2. The age of the most recent common ancestor for BPV-1 variants is estimated to be 1.40 million years (Mya) and for BPV-2 variants, 0.55 Mya. The age of the most recent common ancestor of BPV-1 and BPV-2 is estimated to be 5.34 Mya.

Certain major histocompatibility (MHC) haplotypes are associated with increased risk of sarcoid tumours in horses. The zebras in these parks may have become inbred for the MHC region with increased prevalence for a haplotype, conferring increased risk for sarcoid tumours. Therefore typing system was developed to determine whether or not a high prevalence of sarcoids among zebras is associated with a MHC haplotype. Single strand conformational polymorphism was used to assess the genetic variation in MHC class II genes. The use of DQB and DRB genes demonstrated that genetic variation and sarcoids in the zebras could not be attributed to a specific haplotype.

The developed real-time PCR technique was also applied in the detection of cutaneous papillomavirus in two giraffes (*Giraffa camelopardalis*) which were manifesting cutaneous papillomatosis, in the Kruger National Park and in a fibropapilloma in a sable antelope (*Hippotragus niger*), on a game farm in the Kimberley district, South Africa.

In conclusion, this was the first study to confirm the presence of BPV-1 and -2 DNA in the sarcoid tumours, healthy skin and blood of sarcoid-affected and healthy free-roaming zebras from sarcoid-affected parks. The presence of BPV-1 and -2 DNA in the blood of zebras from parks where sarcoids have not been previously observed was a significant finding.

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