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Prevalence and geographical distribution of *Papio hamadryas papillomavirus 1* (PhPV1) in Kenyan Baboons

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Running title: Prevalence of baboon Papillomavirus in Kenya.

ABSTRACT

Papio hamadryas papillomavirus (PhPV) 1, 2 & 3, are Alphapapillomaviruses that have been detected in Kenyan Olive baboons but the distribution is unknown. Therefore, cervical screening for *PhPV1* was done in baboons from various areas in Kenya using a nested polymerase chain reaction. The prevalence rate was 33 %.

Key words: PhPV-1, Cancer, PCR, distribution

INTRODUCTION.

Human papillomavirus (HPV) is an Alphapapillomavirus (αPV) that is the most common sexually transmitted viral infection and main cause of cervical intraepithelial neoplasia (CIN) and cervical cancer in women [6]. In animals, Bovine Papillomavirus [4] and Cotton Rabbit Papillomavirus [5] have been used as model systems to study the biology of HPV while experimental induction of papillomas and their neoplastic progression has been demonstrated and reproduced in cattle [7] and rabbits [8]. However none of these models recapitulate the natural progression of cervical infection leading over time to neoplastic transformation as seen in HPV-infected women.

In contrast, naturally occurring genital αPVs have been described so far in five non-human primate (NHP) species [13] offering the opportunity for study in an animal model physiologically and phylogenetically closer to humans. Most notably, cervical infection with the potential for neoplastic transformation has been shown in macaques [9, 10, 14] and baboons [3]. Twenty-one naturally occurring NHP αPV have been reported, as reviewed by Rector and Van Ranst [13]. In Olive baboons high-grade (HSIL) and low-grade (LSIL) squamous intraepithelial lesions were associated with infection by PhPV1 [3]. In the pygmy chimp and Colobus monkey, αPV-associated oral epithelial hyperplasia has been reported [15]. As in women, dysplasia or neoplastic transformations are relatively rare outcomes of infection.

The presence of HSIL and LSIL with naturally occurring PhPV1 in Olive baboons *(Papio anubis)* is well known (3) and offers a promising animal model for HPV. The baboon has several advantages and is a unique preclinical model for research in human reproduction [12, 11].

Apart from our study [3], previous efforts to identify αPV in other baboon colonies were unsuccessful [16]. We therefore sought to determine the prevalence of PhPV1 infection in baboons from various areas in Kenya.

Materials and methods: A total of 104 baboons were sampled for this study from March 2013 to January 2015. These were a subset of a total IPR population of approximately 200 post-adolescent female baboons. Criteria used to select animals for sampling consisted of convenience (ex. sedation for veterinary reasons or for other experiments during the study period), sex (females sampled), and weight (post adolescent or > 6.0 kgs, with heaviest being 26.4 kgs).

Ethical approval for this work was given by the IPR Institutional Review committee (IRC/09/13). The animal care and use program at the IPR is based on the Guide for the Care and Use of Laboratory Animals, National Research Council, National Academy Press, Washington D.C., 2011 and in line with the 3R principles. Cervical swabs were collected from each female baboon under Ketamine/Xylazine anesthesia. DNA

extraction was done using the POWERSOIL® DNA Isolation kit (Mo Bio Laboratories Inc., Carlsbad, California, USA). [2].

PCR amplification was done using primers based on the 8000 bp genomic sequence of PhPV1 which was previously sequenced [3] and is publicly available as Gene bank accession number JF304764 (type 1 isolate Mac2085). Primer sequences were as follows: Primary amplification forward primer (Fr2Fout: GGGTATGACGTGAGGCAGTT); Primary amplification reverse primer (Fr1Rout: TACGCAACTTTGGTGGTTCA); Secondary amplification forward primer (Fr2Fin: TGGCATAGGGTTTCATGAGC); Secondary amplification reverse primer (Fr1Rin: TGCAATGTGGCTCAATAAGG)

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Nine *µl* of each of each secondary PCR product was run on a 1.5% agarose gel.

Validation of the nested PCR strategy for this study was performed prior to DNA extraction from sample swabs using samples from animals previously demonstrated as positive or negative for PhPV1 [3]. Sequencing was performed to the products of this validation samples after purification of the amplicon using GENEJET® Genomic DNA Purification Kit (Pittsburgh, Pennsylvania, United States) and was sequenced using Sanger sequencing with the ABI V3.1 Big dye kit on the ABI 3500XL genetic analyzers at Inqaba Company, South Africa.

RESULTS.

The geographic origins of all the animals tested and their respective prevalence of the PhPV1 status is shown in Table 1. A total of 34 of 104 (33 %) baboons were positive for PhPV1 using the nested PCR primers.

The highest percentage of PhPV 1 positive baboons, by body weight, was between 12.0 kgs to 15.9 kgs, corresponding to sexually mature animals. At this weight range, almost 50% were positive to PhPV 1 (Table 2).

DISCUSSION.

This study documents the geographical distribution of PV in Kenyan baboons which was previously unknown. The overall prevalence of PhPV1 infection in this study was 34 /104 (33 %) showing that Kenyan baboons naturally harbor PV.

This current study, also revealed that most infections appeared within the weight range of 12 to 16 kgs. This weight range is consistent with sexual maturity and suggests that this time period is a risk factor for PhPV1 infection. Indeed, baboons menarche occurs between 4 and 5 years of age in wild populations and at about 3.5 years in captivity [1], corresponding to a body weight 8-10 kgs at IPR. This pattern of distribution of HPV has also been observed in women, where HPV prevalence peaks at younger ages (puberty to <25 years) [6].

In conclusion, the findings of this study which showed that there is a natural occurring PV infection, as well as our previous studies showing association of baboon PV with HSIL and LSIL [3], is a strong support for the baboon model in understanding of αPV pathogenesis as well use in future studies for interventional strategies. However, more studies on genomic diversity and establishment of an experimental PV baboon infection model are recommended.

ACKNOWLEDGMENT AND CONFLICT OF INTEREST

There is no Conflict of Interest from among the authors.

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Table 1:

Proportion, Geographic Origins and Papio hamadryas papillomavirus (PhPV1) status of Olive baboons from the Institute of Primate Research, Kenya.

	PhPV1 Negative	PhPV1 Positive	Percentage positive
Laikipia ADC Mutara Ranch	27	10	27
Aberdares, Mahinga	17	8	32
Aberdares, Muringa	5	1	17
Aberdares, Country Club	5	4	44
Yatta NYS Field Unit	5	2	29
Aberdares, Lamuria	4	3	42
Amboseli Jean Altman troops	2	0	0
Colony borne- IPR	2	0	0
Ngurumani	1	1	50
Nairobi National Park	1	4	80
Aberdares, Mweiga	1	1	50
TOTAL	**70**	**34**	**33**

TABLE 2:

Distribution of the percentage of *Papio hamadryas* papillomavirus 1 (PhPV1) positive baboon by body weight at Institute of Primate Research, Kenya.

Weight range	6.0 – 7.9	8.0 - 9.9	10.0 - 11.9	12.0 - 13.9	14.0 – 15.9	16.0 – 17.9	18.0 – 19.9	20.0 – 21.9	22.0 – 23.9	24.0 – 25.9	26.0 – 27.9	Unknown weight[a]
Total Number	4	11	20	31	27	7	1	0	0	0	1	2
Positive	0	1	4	14	13	1	1	0	0	0	0	0
Percentage of positive cases	0	9	20	45	48	14	1	0	0	0	0	0

a. Two baboons caught at Amboseli and released had no weights taken.