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Brief Communication

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Identification of *Felis catus* papillomavirus type 3 within skin neoplasms from four cats.

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Running title: FcaPV-3 and feline skin cancer

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Abstract

Bowenoid *in situ* carcinomas (BISCs) are PV-induced skin neoplasms that are thought to be
42 caused by *Felis catus* papillomavirus (FcaPV) type-2. As BISCs are typically multiple and
can become extensive, they can be difficult to treat. Herein are described four cats that
developed skin neoplasms that contained FcaPV-3 DNA. One cat developed multiple basal
45 cell carcinomas (BCCs), one a BISC with unusual extension into hair follicles, and two cats
each developed a single typical-appearing BISC. All neoplasms contained prominent PV-
induced cell changes and intense p16^{CDKN2a} protein immunostaining. This is the first report of
48 a cat with multiple BCCs and the first evidence that FcaPV-3 may influence the development
of these neoplasms in cats. Additionally, results from these four cats provide evidence that
FcaPV-3 could cause feline skin cancer, albeit less frequently than FcaPV-2. Excision of the
51 typical BISCs and the BCCs appeared curative. While the cat with the unusual BISC was
euthanized due to the large size of the lesion, evidence from these four cats suggest that
skin neoplasms that contain FcaPV-3 DNA may have a less aggressive clinical behavior than
54 those associated with FcaPV-2. A consistent feature of the neoplasms in all four cats was the
presence of prominent basophilic intracytoplasmic inclusion bodies. As these have not been
reported in lesions caused by FcaPV-2, these inclusions may allow differentiation between
57 the different papillomavirus types and could therefore be a useful prognostic feature.

Keywords. Basal cell carcinoma; Bowenoid in situ carcinoma; cat; papillomavirus, skin
60 cancer, viral oncogenesis

Recent studies suggest that papillomaviruses (PVs) cause feline viral plaques and
63 Bowenoid *in situ* carcinomas (BISCs)⁷ and may cause a proportion of cutaneous SCCs in
cats.^{1,18} Currently *Felis catus* papillomavirus (FcaPV) type-2 is thought to cause the majority
of skin disease in cats.^{1,12,15} Herein are described three cats that developed BISCs and one cat
66 that developed multiple feline basal cell carcinomas (BCCs) in which FcaPV-3 was
identified. While a single case of a BISC that contained only FcaPV-3 DNA sequences has
been reported,¹⁰ the development of BISCs containing FcaPV-3 DNA in multiple animals
69 provides evidence of a possible role of this PV type in the development of feline BISCs.
Additionally, this is the first report of multiple BCCs in a cat and the first time FcaPV-3 has
been detected in a neoplasm of this type.

72 Case No. 1 was a 14-year-old male domestic shorthair (DSH) cat that developed three
<1 cm intradermal skin masses on the ventral neck. Excisional biopsy was performed and
histology revealed all were horizontally-orientated elongated masses present within the
75 superficial dermis. One neoplasm had multifocal contiguity with the overlying epidermis. No
contiguity was visible in the other two neoplasms; however, atypical cells were visible
expanding follicular infundibula adjacent to both neoplasms suggesting contiguity may have
78 been present within non-sectioned areas. Neoplastic cells were visible infiltrating from the
tumor into the surrounding dermis and all three neoplasms were classified as BCCs. The
neoplastic cells were arranged in variably-sized aggregates that were divided into nests and
81 trabeculae by a moderate highly cellular fibrovascular stroma (Fig. 1). Palisading of cells was
visible around the periphery of some cell trabeculae and large cystic cavities that contained
only small quantities of eosinophilic cell debris were frequent within the neoplasms.
84 Prominent clefts were visible between the cell trabeculae and the surrounding stroma in one
tumor. The neoplastic cells exhibited mild pleomorphism with the cells generally small dark
and polygonal with small dark nuclei and little cytoplasm. No spindle shaped cells were

87 visible within any of the neoplasms and the neoplasms were not surrounded by a fibrous
pseudocapsule. Prominent within all three neoplasms were cells with enlarged pale foamy
nuclei surrounded by a darkly basophilic elongated intracytoplasmic body that assumed the
90 shape of the nucleus (Fig. 2). Less commonly cells that had a shrunken eosinophilic or
basophilic nucleus that was surrounded by a clear cytoplasmic halo (koilocytes) were visible.
Neoplastic cells did not extend to tissue margins.

93 Due to the histological evidence of PV infection within the BCCs, DNA was extracted
from formalin-fixed tissue as previously described.¹³ To amplify PV DNA the consensus
PCR primers FAP59/64 and MY09/11 were used along with the JMPF/R primers which
96 specifically amplify FcaPV-2 DNA.^{2,6,13} A BISC known to contain FcaPV-2 was used as the
positive control for the FAP59/64 primers while a feline sarcoid containing bovine
papillomavirus type 14 was the positive control for the MY09/11 primers. No template DNA
99 was added to the negative controls. DNA was only amplified from the feline BCCs by the
MY09/11 primers with sequencing subsequently revealing that all three neoplasms contained
FcaPV-3 DNA sequences.

102 Immunostaining to detect p16^{CDKN2a} protein (p16) was performed as previously
reported¹² and revealed intense nuclear and cytoplasmic immunostaining within all the
neoplastic cells within the BCCs. Immunostaining was not visible within the surrounding
105 epidermis or in histologically normal follicles within the sections.

Approximately 6 months after the three BCCs were removed an ulcerated 1.5cm
diameter mass was observed on the ventral neck. Histology revealed a BCC with large
108 numbers of cell nests infiltrating into the surrounding dermis. Papillomavirus-induced cell
changes that included cells with basophilic intracytoplasmic inclusions were prominent and
FcaPV-3 DNA was again the only PV type amplified. The neoplastic cells contained intense
111 nuclear and cytoplasmic p16 immunostaining.

Three apocrine ductular adenomas were also excised from the limbs of this cat at this time. These did not contain histological evidence of PV infection and no PV DNA could be
114 amplified using the FAP59/64, MY09/11 or JMPF/R primers. Additionally, no increased p16 immunostaining was present.

No additional masses have developed in the 1 year following the surgical excision of
117 the BCC from the neck.

Case No. 2 was a 14-year-old male DSH cat that developed roughly symmetrical scabby lesions within both preauricular areas. Over the next 18 months the lesions slowly
120 expanded resulting in a raised plaque that extended from the pinna to the eyes bilaterally. The large size of the lesion prevented excision, but histological examination of a sample of the lesion revealed thickening of the epidermis and underlying follicles. In contrast to the
123 typical appearance of a BISC in which follicular changes are restricted to the infundibulum, thickening was present in the external root sheath extending to the level of the hair bulb (Fig. 3). Cells within the thickened follicles were supported by a minimal fibrous stroma that
126 separated the cells into broad trabeculae and nests. Cells showed palisading along the periphery of the trabeculae and the peripheral cells were smaller and darker than more centrally-positioned cells. Cells within affected areas showed moderate to marked atypia, but
129 were generally polygonal with small dark nuclei and moderate quantities of basophilic cytoplasm. Keratinization was not visible within the thickened follicles. Evidence of PV infection included numerous cells with enlarged pale vesicular nuclei with elongated
132 basophilic cytoplasmic bodies. The lesion was classified as a BISC, although with unusual follicular changes.

Molecular and immunohistochemical analyses were performed as before and revealed
135 that FcaPV-3 was the only PV present and that the cells had intense p16 immunostaining.

Over the following six months the lesions continued to expand and became ulcerated. The cat was euthanized, but a necropsy was not performed.

138 Case Nos. 3 and 4 were both 10-year-old male DSH cats that each developed a single plaque-like scaly mass on the dorsal surface of the paw and nasal planum respectively. Histology of both masses revealed well-demarcated foci of irregular thickening and dysplasia
141 of the epidermis without significant follicular involvement. Papillomavirus-induced cell changes, similar to those in the previous cases, were prominent within the affected epidermis (Fig 4). The only PV detected in the lesions was FcaPV-3 and both lesions exhibited intense
144 p16 immunostaining. Both were consistent with BISCs, although the basophilic elongated intracytoplasmic inclusions were not typical. Neither lesion recurrence nor additional lesion development has been observed in the 12 months following excision.

147 Most skin disease due to PVs in cats is currently thought to be caused by FcaPV-2.⁷ While a single case in which FcaPV-3 was the only PV type detected in a feline BISC has been reported,¹⁰ the results from the four currently reported cats provide **additional** evidence
150 that FcaPV-3 **could** cause skin neoplasia in cats. While the proportion of PV-induced skin lesions that **contain FcaPV-3 DNA in** cats is unknown, FcaPV-2 DNA has been consistently detected in previous large series of feline viral plaques and BISCs,^{13,14} suggesting that
153 FcaPV-3 is comparatively rarely associated with skin disease in cats.

The lesions observed in the four cats ranged from the presence of mild BISC-like changes with little follicular involvement to the development of a BCC within the dermis.
156 Interestingly Case No. 2 had lesions that included changes to both the epidermis as well as the deep segment of the hair follicle. The apparent ability of FcaPV-3 to infect cells within the epidermis and deeper within the follicles has not been previously reported and may
159 explain why this PV type **can be present in** both BISCs and BCCs. In contrast, FcaPV-2 appears to be restricted to infecting cells in the surface epidermis and follicular infundibulum

and has not been associated with the development of BCCs in cats.⁴ As PVs tend to be highly
162 specific in the cells that they are able to infect,¹⁶ it is perhaps unsurprising that FcaPV-3 and
FcaPV-2 have small differences in the range of cells that they can infect.

Whether lesions **that contain FcaPV-3 DNA** have a different clinical behavior to those
165 associated with FcaPV-2 is currently unknown. However, cats with BISCs, presumably due
to FcaPV-2, typically develop multiple lesions that are often too numerous to surgically
excise.³ In contrast, none of the currently reported cats had greater than four lesions and
168 single lesions were observed on two cats. Furthermore, in contrast to cats infected by FcaPV-
2 that often rapidly develop additional BISCs,³ surgical excision of the lesions **that contained**
FcaPV-3 DNA appeared to be curative in three of the four presently described cats. The BISC
171 in Case No. 2 did result in euthanasia suggesting that lesions **that contain FcaPV-3 DNA** can
potentially be progressive and involve large areas of skin. However, as the lesions in this cat
had been present for 18 months prior to diagnosis, it is possible a more prompt intervention
174 could have been curative. While additional cases are required, there is accumulating evidence
that FcaPV-3 may cause skin neoplasms that have a less aggressive clinical course than those
associated with FcaPV-2.

177 The histological appearance of the lesions in the four cats was diverse. However, all
contained cells with prominent basophilic intracytoplasmic inclusions. Such inclusions have
been previously been reported in a BISC that contained only FcaPV-3 sequences.¹⁰ However,
180 basophilic intracytoplasmic inclusions have not been reported in lesions associated with
FcaPV-2. Therefore, histology is likely to enable differentiation between FcaPV-2 and
FcaPV-3 and, if prognostic differences between lesions caused by the two different PV types
183 are proven, these basophilic inclusions could be an easily-observed prognostic feature. As
FcaPV-2 and FcaPV-3 are classified within different genera^{5,9}, it is unsurprising that infection

by these two PV types produce different papillomavirus-induced cell changes within the
186 lesions.

The neoplasms observed in case No. 1 were classified as BCCs. While differentiation
of a BCC from a follicular or apocrine gland neoplasm can be difficult, features that support a
189 basal cell origin in this case include the horizontal orientation of the masses within the
dermis. Additionally, the presence of prominent clefts between the cell trabeculae and the
surrounding stroma has been reported to be a diagnostic feature of BCCs in people.⁴
192 Furthermore the absence of spindle-shaped cells or a fibrous pseudocapsule within the
neoplasms is more consistent with a BCC than a neoplasm of apocrine gland or follicular
origin.⁴ Basal cell carcinomas with histological evidence of PV infection have been
195 previously described and sequences from a novel PV type were reported in a feline BCC.^{4,11}
However, this is the first report of FcaPV-3 being identified within a feline BCC and this
report increases the spectrum of skin neoplasms that could be influenced by this PV type. To
198 the authors' knowledge, multiple BCCs have not been previously reported in a cat. As BCCs
are considered to be rare neoplasms of cats, the development of three BCCs on the same
animal suggests a predisposing factor was likely to have influenced tumor development.
201 While it cannot be definitively confirmed that infection by FcaPV-3 was the factor that
predisposed to multiple neoplasm development in this cat, the presence of PV-induced cell
changes supports the hypothesis of a causal association between FcaPV-3 and the multiple
204 BCCs in this cat.

As PVs have co-evolved with their hosts over a long period of time, most PV
infections are asymptomatic.¹⁷ Therefore, the amplification of PV DNA from a lesion does
207 not prove causality. In cats, FcaPV-2 is generally considered to cause BISCs due to the
presence of PV-induced cell changes within a proportion of tumors, the more frequent
detection of FcaPV-2 in BISCs than normal skin, the higher FcaPV-2 viral loads in BISCs

210 than normal skin, and the consistent detection of FcaPV-2 RNA in BISCs, but not normal
skin samples.^{7,13,17,18} In addition, a role of FcaPV-2 in BISC development is supported by the
increased p16 immunostaining observed in these lesions.⁸ This protein increases due to the
213 consistent action of the PV E7 protein degrading retinoblastoma protein which, in turn,
increases p16 within a cell.¹² In the presently reported cats, a role of FcaPV-3 in lesion
development was supported by the presence of histologically-detectible PV-induced cell
216 changes within the neoplastic cells. In addition, FcaPV-3 DNA was detected in lesions from
all 3 cats, but this PV was not identified in non-lesional skin from one of the affected cats and
this PV has been rarely detected in previous studies investigated the presence of PVs on the
219 skin of cats.⁸ Furthermore, the presence of intense p16 immunostaining in lesions from all
four cats is consistent with PV infection influencing their development. While the specificity
of p16 for a PV etiology is unknown in cats, increased p16 immunostaining was not present
222 in the apocrine tumors from Case No 1 that did not contain histological evidence of PV
infection or amplifiable PV DNA sequences. While these results support a role of FcaPV-3 in
lesion development, it is not possible to definitively differentiate between FcaPV-3
225 influencing neoplasm development and FcaPV-3 being present due to a more permissive
environment.

Evidence from these four cats suggests that FcaPV-3 could cause a proportion of
228 feline skin cancers. Compared to FcaPV-2, FcaPV-3 may be able to infect cells deeper in the
follicle and a proportion of feline cutaneous BCCs may be caused by FcaPV-3 infection.
Lesions associated with FcaPV-3 contain basophilic intracytoplasmic inclusions and these
231 inclusions are not present in lesions associated with FcaPV-2. While additional cases are
required, FcaPV-3 may cause neoplasms with a less aggressive clinical course than those
associated with FcaPV-2 and recognition of these inclusions may be an important prognostic
234 feature.

Declaration of conflicting interests,

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References

- 246 1 Altamura G, Corteggio A, Pacini L, et al.: 2016, Transforming properties of *Felis catus*
papillomavirus type 2 E6 and E7 putative oncogenes in vitro and their transcriptional
activity in feline squamous cell carcinoma in vivo. *Virology* 496:1-8.
- 249 2 Forslund O, Antonsson A, Nordin P, et al.: 1999, A broad range of human papillomavirus
types detected with a general PCR method suitable for analysis of cutaneous tumours
and normal skin. *J Gen Virol* 80:2437-2443.
- 252 3 Gill VL, Bergman PJ, Baer KE, et al.: 2008, Use of imiquimod 5% cream (Aldara) in cats
with multicentric squamous cell carcinoma in situ: 12 cases (2002-2005). *Vet Comp
Oncol* 6:55-64.
- 255 4 Gross TL, Ihrke PJ, Walder EJ, et al.: *Skin diseases of the dog and cat: clinical and
histopathologic diagnosis*, 2nd edition ed. pp. 567-589. Blackwell Science, Oxford,
United Kingdom, 2005
- 258 5 Lange CE, Tobler K, Markau T, et al.: 2009, Sequence and classification of FdPV2, a
papillomavirus isolated from feline Bowenoid in situ carcinomas. *Vet Microbiol*
137:60-65.
- 261 6 Manos MM, Ting Y, Wright DK, et al.: 1989, The use of polymerase chain reaction
amplification for the detection of genital human papillomaviruses. *Cancer Cells*
7:209-214.
- 264 7 Munday JS. 2014, Papillomaviruses in felids. *Vet J* 199:340-347.
- 264 8 Munday JS, Aberdein D. 2012, Loss of retinoblastoma protein, but not p53, is associated
with the presence of papillomaviral DNA in feline viral plaques, Bowenoid in situ
carcinomas, and squamous cell carcinomas. *Vet Pathol* 49:538-545.
- 267 9 Munday JS, Dunowska M, Hills SF, et al.: 2013, Genomic characterization of *Felis catus*
papillomavirus-3: a novel papillomavirus detected in a feline Bowenoid in situ
carcinoma. *Vet Microbiol* 165:319-325.
- 270 10 Munday JS, Fairley R, Atkinson K. 2016, The detection of *Felis catus* papillomavirus 3
DNA in a feline bowenoid in situ carcinoma with novel histologic features and benign
clinical behavior. *J Vet Diagn Invest* 28:612-615.
- 273 11 Munday JS, French A, Thomson N. 2017, Detection of DNA sequences from a novel
papillomavirus in a feline basal cell carcinoma. *Vet Dermatol* 28:236-e60.

- 276 12 Munday JS, French AF, Peters-Kennedy J, et al.: 2011, Increased p16CDKN2A protein
within feline cutaneous viral plaques, bowenoid in situ carcinomas, and a subset of
invasive squamous cell carcinomas. *Vet Pathol* 48:460-465.
- 279 13 Munday JS, Kiupel M, French AF, et al.: 2008, Amplification of papillomaviral DNA
sequences from a high proportion of feline cutaneous in situ and invasive squamous
cell carcinomas using a nested polymerase chain reaction. *Vet Dermatol* 19:259-263.
- 282 14 Munday JS, Peters-Kennedy J. 2010, Consistent detection of *Felis domesticus*
papillomavirus 2 DNA sequences within feline viral plaques. *J Vet Diagn Invest*
22:946-949.
- 285 15 O'Neill SH, Newkirk KM, Anis EA, et al.: 2011, Detection of human papillomavirus
DNA in feline premalignant and invasive squamous cell carcinoma. *Vet Dermatol*
22:68-74.
- 288 16 Sundberg JP, Van Ranst M, Montali R, et al.: 2000, Feline papillomas and
papillomaviruses. *Vet Pathol* 37:1-10.
- 291 17 Thomson NA, Dunowska M, Munday JS. 2015, The use of quantitative PCR to detect
Felis catus papillomavirus type 2 DNA from a high proportion of queens and their
kittens. *Vet Microbiol* 175:211-217.
- 294 18 Thomson NA, Munday JS, Dittmer KE. 2016, Frequent detection of transcriptionally
active *Felis catus* papillomavirus 2 in feline cutaneous squamous cell carcinomas. *J*
Gen Virol 97:1189-1197.

297 Figure Legends

Figure 1. Basal cell carcinoma, Case No. 1. The mass consists of a horizontally elongated
proliferation of neoplastic epithelial cells arranged in nests and trabeculae surrounding
300 multiple cystic cavities. Neoplastic cells are visible infiltrating into the surrounding dermis.
Hematoxylin and Eosin.

303 Figure 2. Basal cell carcinoma, Case No. 1. Numerous cells that contain enlarged vesicular
nuclei and prominent elongate basophilic intracytoplasmic bodies are visible within the
neoplasm (arrows). Hematoxylin and Eosin. The neoplastic cells consistently contain intense
306 cytoplasmic and nuclear immunostaining using antibodies against p16^{CDKN2a} protein (inset).

Figure 3. Bowenoid *in situ* carcinoma, Case No. 2. Consistent with previous reports of feline
309 Bowenoid *in situ* carcinomas, thickening and the presence of dysplasia is visible within the
epidermis. However, in contrast to typical Bowenoid *in situ* carcinomas in which follicular

changes are confined to the infundibulum, thickening is present within the external root
312 sheath extending close to the hair bulb. Hematoxylin and Eosin.

Figure 4. Bowenoid *in situ* carcinoma, Case No. 3. The epidermal changes observed are
315 considered typical for a Bowenoid *in situ* carcinoma. However, cells with enlarged vesicular
nuclei and prominent basophilic elongate cytoplasmic inclusions, similar to those observed in
the basal cell carcinoma, are visible (arrows). Hematoxylin and Eosin.

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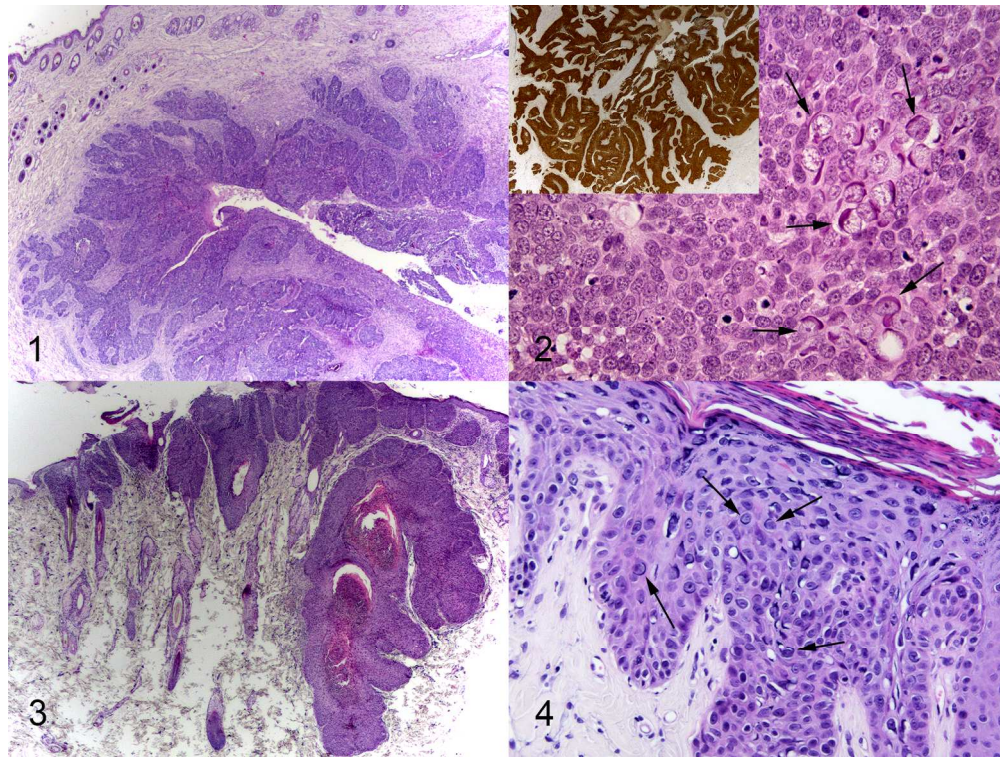


Figure 1. Basal cell carcinoma, Case No. 1. The mass consists of a horizontally elongated proliferation of neoplastic epithelial cells arranged in nests and trabeculae surrounding multiple cystic cavities. Neoplastic cells are visible infiltrating into the surrounding dermis. Hematoxylin and Eosin.

Figure 2. Basal cell carcinoma, Case No. 1. Numerous cells that contain enlarged vesicular nuclei and prominent flattened basophilic intracytoplasmic bodies are visible within the neoplasm (arrows). Hematoxylin and Eosin. The neoplastic cells consistently contain intense cytoplasmic and nuclear immunostaining using antibodies against p16CDKN2a protein (inset).

Figure 3. Bowenoid in situ carcinoma, Case No. 2. Consistent with previous reports of feline Bowenoid in situ carcinomas, thickening and the presence of dysplasia is visible within the epidermis. However, in contrast to typical Bowenoid in situ carcinomas in which follicular changes are confined to the infundibulum, thickening of the deep segments of the follicles is also visible. Hematoxylin and Eosin.

Figure 4. Bowenoid in situ carcinoma, Case No. 3. The epidermal changes observed are considered typical for a Bowenoid in situ carcinoma. However, cells with enlarged vesicular nuclei and prominent basophilic flattened cytoplasmic inclusions, similar to those observed in the basal cell carcinoma, are visible (arrows). Hematoxylin and Eosin.