



NOTE

Internal Medicine

Detection of *Felis catus* papillomavirus type 2 within multicentric basosquamous carcinoma in a domestic cat

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ABSTRACT. A 12-year-old neutered male domestic shorthair cat was presented with a 3-year history of multiple nonpruritic, ulcerated, plaque-like skin lesions but no other clinical signs. A systemic examination revealed mild lymphadenopathy. Histopathologic analysis of the skin lesions revealed multicentric basosquamous carcinoma (BSC). Immunohistochemical analysis, PCR, and sequencing detected *Felis catus* papillomavirus type 2 (FcaPV-2) within the tumors. As BSC is rare in cats, clinical behavior has not been established. To our knowledge, this is the first case report to demonstrate detection of FcaPV-2 within a BSC in a domestic cat.

KEY WORDS: basosquamous carcinoma, cat, papillomavirus

Basosquamous carcinomas (BSCs) are rarely reported in cats [4] and are a rare skin cancer entity in humans [37]. BSCs have histopathological characteristics of both basal cell carcinomas (BCCs) and squamous cell carcinomas (SCCs) [8]. In humans, BSCs are clinically and morphologically similar to BCCs, but tumor behavior may be more aggressive than that of BCCs, and distance metastasis is also possible [11]. However, human BSCs are rare, and there is still controversy concerning the histologic definition and clinical behavior [11, 34]. BSC metastasis in cats has not yet been reported, and BSCs are considered a low grade malignancy in domestic animals [9]. Tumor behavior and the true incidence of BSCs have not been established in cats as they have been in humans [10].

Papillomaviruses (PVs) are small, non-enveloped, circular, double-stranded DNA viruses that infect the stratified squamous epithelium of mammals [29]. Many papillomaviral infections in cats do not result in skin lesions [35] because the immune system prevents the PV from altering epithelial cell regulation [7]. However, when an epithelial cell change occurs, skin lesions such as papillomas, viral plaques, Bowenoid *in situ* carcinomas (BISCs), invasive SCCs, and feline sarcomas may occur due to epithelial hyperplasia [1, 20, 25, 29, 36]. PVs were detected more frequently in cats with BISCs and SCCs than cats with non-neoplastic skin lesions [30]. Recently, it was reported that BCC is also involved [23]. Five *Felis catus* papillomavirus (FcaPV) types have been fully sequenced, and it is believed that FcaPV-2, -3, and -4 are associated with skin cancer [29]. However, FcaPV-2 can be detected in the skin of healthy queens and kittens [35], and the cancer-causing mechanism of FcaPVs is not known [29]. The relationship between BSCs and PVs is still unknown in cats. PVs have been detected in BSCs of a bat and humans [3, 32, 39], but not of cats. Here, we report not only the clinical features and histopathological characteristics, but also the immunohistochemistry (IHC) and polymerase chain reaction (PCR) methodology and results of the detection of FcaPV in a cat with multicentric BSC.

A 12-year-old neutered male domestic shorthair cat weighing 6.7 kg was referred to a referral animal hospital for diagnosis of a 3-year history of multiple nonpruritic skin lesions. The cat had a history of antibiotic treatment and was unresponsive to treatment.

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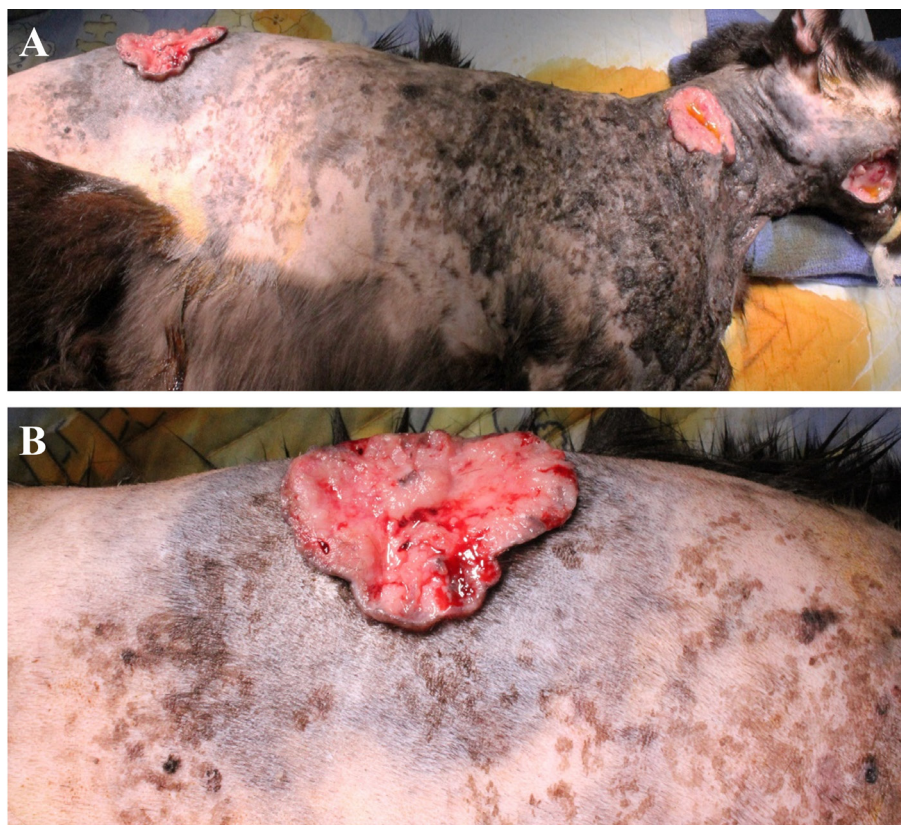


Fig. 1. Multiple skin lesions at initial presentation (A, B). Hyperpigmentation, crust, and ulcerations occurred on the cheek, neck, and back, and irregular horn-like pigmented plaques occurred predominantly on the neck and shoulder. Note the raised, ulcerated lesions at multiple sites.

No additional clinical signs were detected in 3 years. On physical examination, there were multiple large ulcerations around the cheek, neck, and back, and multiple darkly hyperpigmented plaques less than 1 cm in diameter diffusely on the neck, shoulder, and ventrum (Fig. 1). All lesions were in haired areas and on pigmented skin. The slightly raised ulcerations were approximately 3 to 8 cm in diameter. Systemic screenings (blood analysis, thoracic radiography, and abdominal ultrasonography) were conducted for diagnosis. A complete blood count revealed neutrophilia (19.28×10^9 cells/l; reference range: $1.15\text{--}10.29 \times 10^9$ cells/l). A blood biochemical profile revealed mild hypoalbuminemia (2.4 g/dl, reference range: 2.6–3.9 g/dl) and hyperglobulinemia (6.7 g/dl, reference range: 3.0–5.9 g/dl). Tests for feline leukemia virus (FIV) and feline immunodeficiency virus (FeLV) were negative (SNAP FIV/FeLV Combo Test, IDEXX Reference Laboratories, Westbrook, ME, U.S.A.). Thoracic radiography revealed no evidence of metastases. Abdominal ultrasonography indicated a mildly enlarged right medial iliac lymph node (5.1 mm) concurrent with decreased parenchymal echogenicity (Fig. 2). Otherwise, the hepatic and splenic parenchyma were normal.

All ulcerations on the skin were excised and histopathologically examined. The final diagnosis was multicentric BSC of the skin. Multiple small biopsies had infiltrative, nonencapsulated, densely cellular neoplasms in the epidermis to the deep dermis infiltrating into the subcutis. The neoplasm was composed of neoplastic basaloid epithelial cells and a substantial portion of squamous epithelial cells (Fig. 3). Neoplastic basaloid cells were arranged in solid nests and cords in a disorganized pattern and were supported by small amount of a fine fibrovascular stroma. Neoplastic basaloid cells had scant eosinophilic cytoplasm with a round to oval nucleus. Neoplastic squamous epithelial cells had moderate to abundant cytoplasm with large round nuclei. The nucleus had finely to coarsely stippled chromatin and 1–2 distinct nucleoli. The mitotic index was 12 per 10 high-power fields. Neoplastic squamous epithelial cells had gradual and abrupt squamous differentiation with central keratin pearls mixed with some neutrophils. Multiple small areas of necrosis, scattered lymphocytes, plasma cells, and hemorrhage were present at the periphery. IHC using a polyclonal antibody against bovine papillomavirus (Papillomavirus Antibody, EastCoast Bio, North Berwick, ME, U.S.A.) confirmed the presence of PV antigen within the tumor tissues (Fig. 3). A conjugated anti-goat IgG (HRP-conjugated Rabbit Anti-Goat IgG H&L, Abcam plc, Cambridge, U.K.) was used as the secondary antibody. Cytoplasmic immunostaining revealed strong reactivity on neoplastic cells within the BSC, supporting the presence of papillomavirus in the tumors. To determine the type of FcaPV associated with these tumor lesions, DNA was extracted from dewaxed formalin-fixed 10- μ m sections of the histological samples as previously described [26]. The five previously mentioned sets of PCR primers (FcaPV-1, -2, -3, -4, and -5) were used to amplify DNA from tumor tissues in this study [18, 22]. All five primer sets were used at a concentration of 20 pM/Rx. DNA was amplified using the following protocol: 95°C for 5 min followed by 45 cycles of 95°C for 30 sec and 60°C for 30 sec. Amplified

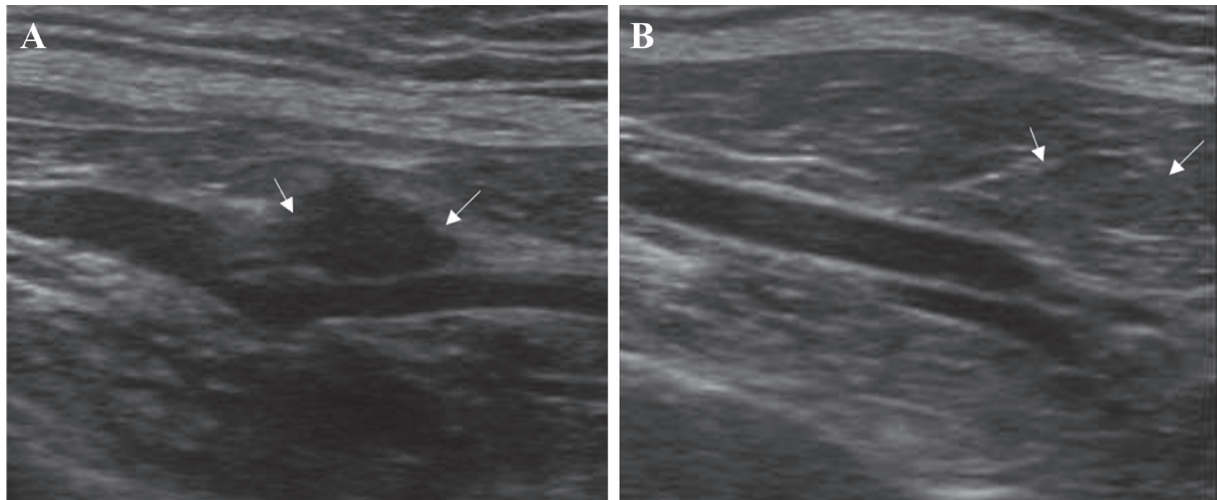


Fig. 2. The right medial iliac lymph nodes (A, between white arrows) of this patient showed a homogeneous hypoechoic echotexture compared to the unaffected left side (B, between white arrows). This lymph node had a maximum length of 1.01 cm and was easy to locate, and had a short/long ratio of 0.6 according to measurements obtained on the dorsal/longitudinal approach.

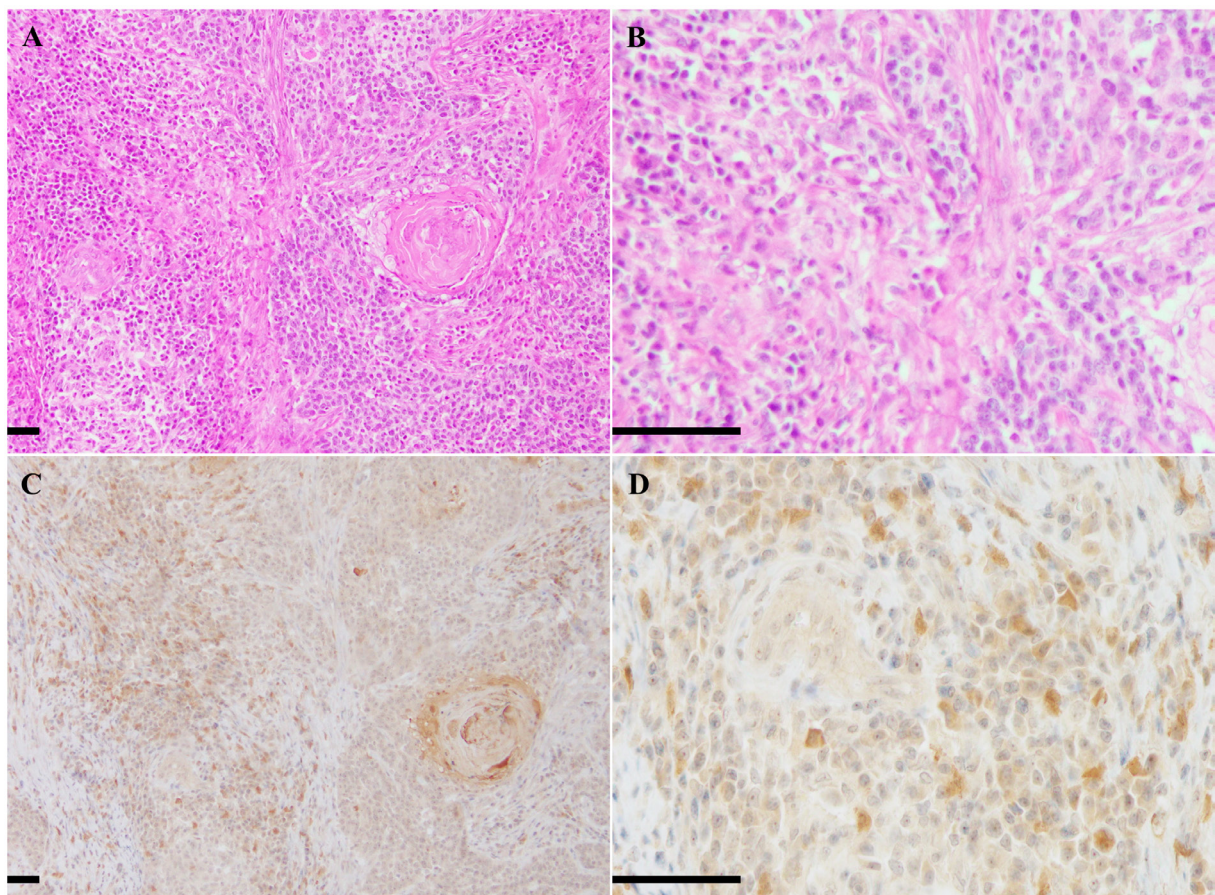


Fig. 3. Histopathological and immunohistochemical images of feline basosquamous carcinoma. Densely cellular neoplasm was composed of neoplastic basaloid and squamous epithelial cells. Basaloid neoplastic cells were arranged in solid nests and cords in a disorganized pattern and were supported by fine fibrovascular stroma. Multiple central keratin pearls had been infiltrated by inflammatory cells (A). Neoplastic basaloid cells had scant eosinophilic cytoplasm with a round to oval nucleus. Neoplastic squamous epithelial cells had moderate to abundant eosinophilic cytoplasm (B). Intense cytoplasmic immunostaining using anti-bovine papillomavirus antibodies was visible within the neoplastic cells (C, D). Bar=50 μ m.

fragments were visualized using an automatic electrophoresis analyzer (QIAxcel, QIAGEN GmbH, Hilden, Germany). FcaPV-2 DNA was the only type amplified from the tissue samples. Negative and positive controls were not included. Amplified DNA was purified and sequenced using an ABI 3130xl Genetic Analyzer (Applied Biosystems Inc., Foster City, CA, U.S.A.) as previously described [26]. Tumor samples contained the same DNA sequence that most closely resembled FcaPV-2, with 99% similarity over the 142-bp length compared to the known sequence available in GenBank (EU796884.1). The patient recovered rapidly after surgery. There was no recurrence until 2 months postoperatively. After that, follow-up was lost.

The patient was diagnosed with BSC. BSCs are rarely seen in cats [4] and little is known about the true incidence, tumor behavior, or prognosis. In humans, BSCs are considered to be more aggressive than BCCs [11], and are reported to have higher recurrence and metastasis rates compared to BCCs [14, 17]. However, controversial results have been reported in recent years [11, 14]. This patient showed no aggressive tumor behavior and no clear metastases. However, due to the presence of mild medial iliac lymphadenopathy on abdominal ultrasonography, the possibility of metastasis cannot be completely eliminated. The lymph nodes were very small, and it was impossible to perform cytology or histopathology. The medial iliac lymph node was very small, so the possibility of inflammation or reactive lymphadenopathy could be considered. The clinical behavior of this tumor was similar to that of feline BISCs. There are indolent growth variants of BCCs in humans [5], which may also be similar.

The histological definition of human BSC is still controversial [34], but most physicians recognize it as a cancer that contains both BCC and SCC with a transition zone of mixed differentiation [6, 16]. Clinically, BSCs are considered to be a stage in a process in which a BCC differentiates into a SCC. Conversely, a BSC cannot differentiate from a BCC or SCC [13]. This tumor has been referred to as a BSC, metatypical BCC, and BCC with squamous differentiation [34]. In humans, BSCs are clinically and morphologically close to BCCs but are more aggressive, and it is believed that distant metastasis is possible [11]. However, BSCs are rare in humans; the incidence is reported to be 1.2 to 2.7% of all BCCs [8], and these results are still controversial in humans as they were reported in a few retrospective series. Feline BSCs rarely metastasize, but occasional recurrence has been reported [4]. In fact, the tumor morphology of this patient is very similar to human BSCs [2], but the tumors were not clinically aggressive. More than 3 years passed without clinical symptoms or metastasis. In this study, the patient may have a favorable prognosis.

This patient had many non-ulcerated, darkly hyperpigmented plaques. These plaques are likely to be viral plaques caused by PVs. Feline viral plaques are mildly raised, hairless, less than 1 cm in diameter and often pigmented [38]. In addition, viral plaques show a slow increase in size and number or may persist without progressing [29]. The plaques of this patient also had similar characteristics. The viral plaques in cats are known to be mainly caused by FcaPV-2 [28]. Unfortunately, in this patient, the plaques were not examined and the viral plaques cannot be completely concluded. These plaques could not be resected in this study because they were diffused widely. There was a case of a viral plaque in a dog that was treated with a carbon dioxide laser [12], but in cats it must be performed before the plaque becomes ulcerated. In humans, the method of BSC resection with a wider surgical margin is used, rather than the methods for BCC or SCC resection [13].

In this patient, FcaPV-2 was detected in the tumor. To the best of the author's knowledge, this is the first case report of FcaPV-2 found in feline BSC. Histopathologic examination of this patient revealed no PV-induced cell changes such as koilocytes [19] seen with PV infection but positive for immunostaining. PVs are a causative agent of skin neoplasia in humans [31], dogs [15, 27], and cats [1, 21, 36]. When PVs infect basal cells, the infection forms episomes in the cell [33] to provide a reservoir of infection, which affects epithelial cell regulation and causes skin lesions. It is reported that FcaPV-2 primarily induces BISCs [26] and SCCs [24]. The frequencies of FcaPV-3 and -4 are low, but can be related to skin cancer [29]. It is assumed that PVs will play a role in tumor development of human BSCs [3, 39]. However, in this case, the presence of FcaPV-2 in the tumor tissue does not mean that the virus has caused the tumor. Because FcaPV-2 can also be found in healthy feline skin [35]. Further investigation is needed to determine the role of FcaPV-2 in the development of feline BSC.

This is a rare case of reporting that the feline BSC appears to be multicentric. Although detection of FcaPV-2 in other feline skin cancers has been reported, to the author's knowledge, this is the first case report of FcaPV-2 detected in feline BSC. We believe that the results of this case report will be helpful in further studies of the diagnosis and the relevance of PVs in feline skin cancer.

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