

Papillomavirus associated skin lesions in a cat seropositive for feline immunodeficiency virus

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ABSTRACT

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A cat was presented with skin lesions consisting of slightly raised pigmented plaques, 2–7 mm in diameter with a rough slightly verrucous surface. Histologically these lesions were identified as papillomas. A papillomavirus infection was demonstrated: virus-like particles were present in the nuclei of cells within the lesions, and staining with an anti-bovine papillomavirus (BPV-1) antibody was obtained. An infection with feline immunodeficiency virus was diagnosed in this cat; this condition had probably enhanced the development of papillomas. This is the first report of a papillomavirus infection in a cat in Europe.

INTRODUCTION

Papillomaviruses, members of the Papovaviridae family, infect many species of mammals and birds (Sundberg, 1987). They cause benign cutaneous and mucosal proliferations that are usually self-limiting and only rarely progress to malignant tumors (Sundberg and O'Bannion, 1989). Most papillomaviruses are host species specific; all members of the genus share at least one antigenic determinant (Sundberg et al., 1984). Studies of papillomatous and hyperplastic lesions in various animal species have been published

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(Sundberg and O'Bannion, 1989; Sironi et al., 1990) but papillomaviral antigen was not demonstrated in lesions from cats until recently. Now two feline cases of epidermal hyperplasia have been described which proved to be related to papillomavirus infection (Carney et al., 1990).

Papillomas are more prevalent in animals and man under conditions that impair T cell functions (Duncan et al., 1975; Alloub et al., 1989; Sundberg and O'Bannion, 1989; Matorras et al., 1991); since infection with feline immunodeficiency virus (FIV) is such a condition (Pedersen et al., 1987), papillomas could be expected in seropositive cats.

In the present report we describe warts as a consequence of a papillomavirus infection in a 6 year old cat that was also infected with FIV. It is the first observation of a papillomavirus infection in a cat in Europe.

MATERIALS AND METHODS

Specimens

Dermal tissue samples were taken from a non-pedigree, 6 year old, castrated male cat with multiple skin lesions consisting of slightly raised, pigmented plaques. The clinical history of the cat was recorded and a blood sample collected for serology. A second biopsy sample for electron microscopy (EM) was taken two weeks after the animal had been first presented.

A full-thickness biopsy of an entire lesion with adjacent normal tissue was taken for histopathological examination and immediately placed in 10% neutral buffered formalin. The sample was routinely processed and embedded in paraffin. Sections were stained with haematoxylin and eosin (H & E), von Gieson stain and the periodic acid Schiff (PAS) stain. Small pieces of the second biopsy sample were placed in 2.5% cacodylate-buffered glutaraldehyde for EM, postfixed in 2% osmium tetroxide, dehydrated and embedded in epoxy resin (Durcupan, Fluka). Ultrathin sections were made using a Reichert Om U2 ultramicrotome using a Diatome diamond knife, stained with uranyl acetate-lead citrate and examined with a Philips EM 200 electron microscope at a primary magnification of 26 000 \times .

Immunohistochemistry

For immunohistochemical examination a commercial rabbit anti-bovine papillomavirus (BPV-1) antibody (DAKO; catalogue No. B580) was used at a 1:800 dilution; the preparation is known to react with papillomavirus-specific common structural antigens. Antigen was demonstrated using a modified alkaline phosphatase-antiphosphatase (APAAP) method described elsewhere (Sironi et al., 1990; Condell et al., 1984); sections were counterstained with haematoxylin. Control sections using normal rabbit serum instead of the anti BPV-1 serum were included. Positive controls consisted of paraffin-embedded material from a human genital wart in which papilloma-

virus DNA had been evidenced by in situ hybridization. From nine other cats aged between 3 months and 13 years, sections of dermal samples diagnosed as papillomatous lesions by histology were examined in the APAAP test; the material had been collected in the past 2 years.

FIV serology

An immunofluorescence assay (IFA) was performed on FIV-infected CrFK cells using sera diluted 1:20 in PBS as described before (Yamamoto et al., 1988). The serum was also tested at a 1:50 dilution in a western blot with gradient purified FIV as explained elsewhere (Egberink et al., 1989). A test for feline leukemia virus (FeLV) antigen in blood samples was performed using a commercial kit (Clin-Ease test, Synbiotics) according to the manufacturer's instructions.

RESULTS

Two months before developing the papillomatous lesions described in this report the cat had been presented with signs of general illness, hair loss, upper respiratory tract infection, and otitis externa. A fungal infection was diagnosed by culturing the organism (*Microsporum canis*) and treated with griseofulvine given orally for 3 weeks. To cure the otitis an unguent containing an antibiotic was given. Subsequently skin lesions were detected which consisted of slightly raised pigmented plaques with a rough, slightly verrucous surface, they measured 2–7 mm in diameter (Fig. 1). Single lesions were more elongated. The wart-like lesions were located on the head, neck and dorsal part of thorax and abdomen.

Histological examination revealed pigmented hyperplastic epidermal plaques, which were sharply demarcated from the unpigmented normal skin. The hair follicles abutting from the plaques were normal. The epidermal hyperplasia was characterized by acanthosis, hypergranulocytosis with orthokeratotic hyperkeratosis and elongated rete ridges (Fig. 2). The hyperplastic epidermal plaques showed marked pigmentation throughout all layers, and in the superficial dermis focal accumulations of melanophages were present. No inflammatory cells were observed, neither in the plaques nor in the adjacent stroma. In the stratum spinosum, often also in the stratum basale, ballooning degeneration of the cells with a hyalin pale blue aspect of the cytoplasm was seen; also a solitary necrotic keratinocyte was present. In the upper part of the stratum spinosum and in the stratum granulosum solitary cells with large amphophilic, ill-defined but probably intranuclear inclusions were seen (Fig. 3). Electron microscopy revealed large numbers of particles with a circular outline and an approximate diameter of 50 nm in the nucleoplasm of keratinized cells in the superficial epithelial strata of the plaques (Fig. 4). Most particles were electron dense, only a few showing an electron-lucent center. Paracryst-

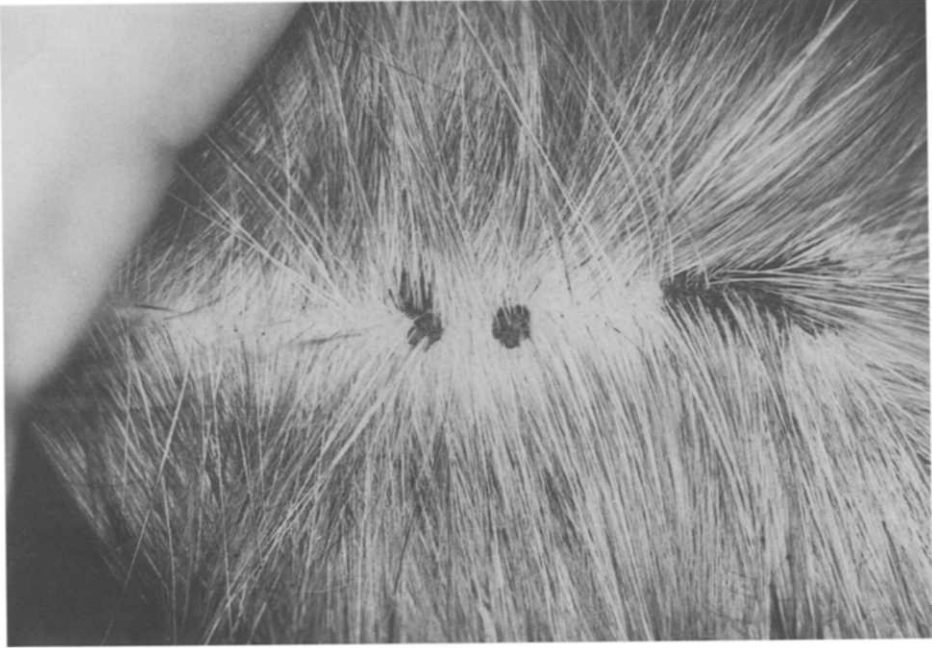


Fig. 1. Typical skin lesion on the dorsal part of the abdomen. Pigmented, slightly raised plaques with a rough surface can be seen.

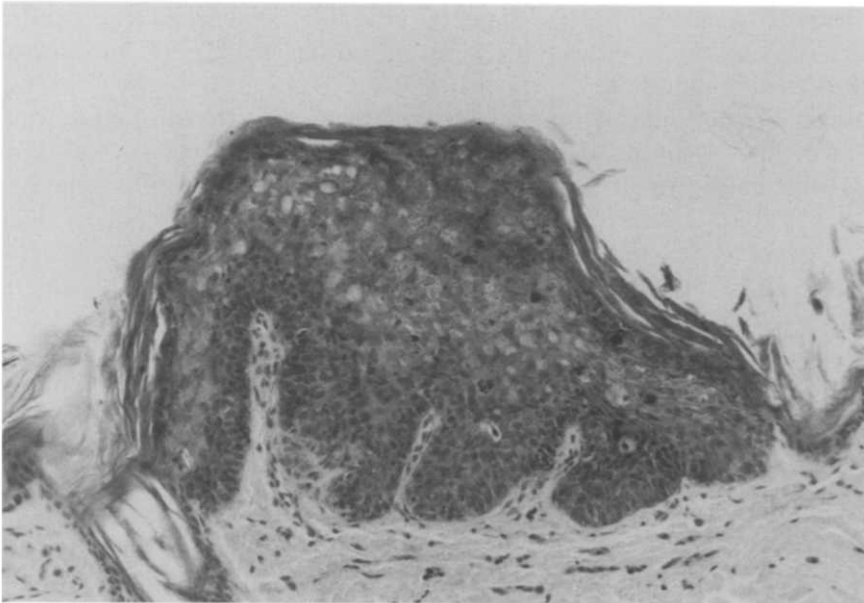


Fig. 2. Hyperplastic epidermal plaque with acanthosis, hypergranulocytosis, hyperkeratosis and elongated rete ridges. H & E stain; 150 \times .

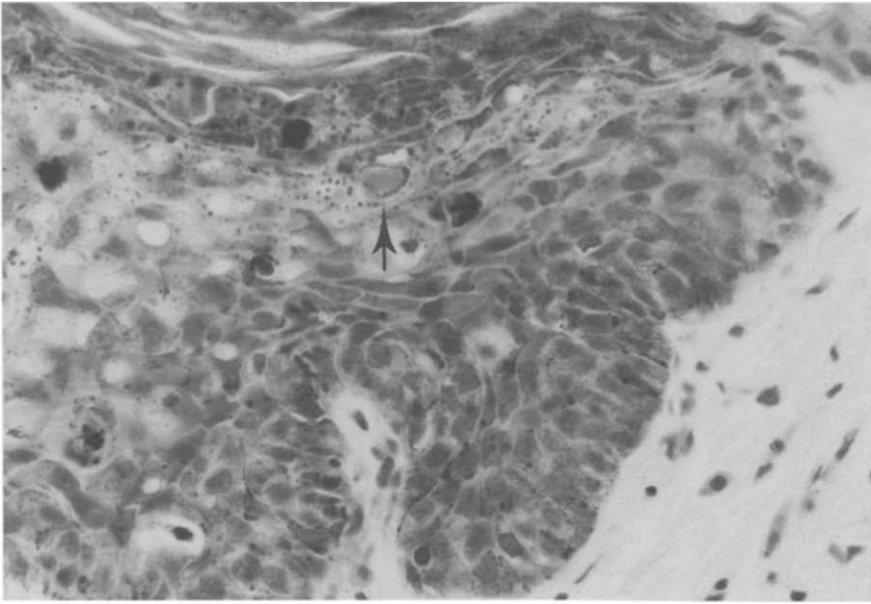


Fig. 3. Hyperplastic epidermal plaque with hyperpigmentation, ballooning degeneration and hypergranulocytosis. In the superficial layers inclusion bodies (arrow) are present. H & E stain; 420 \times .

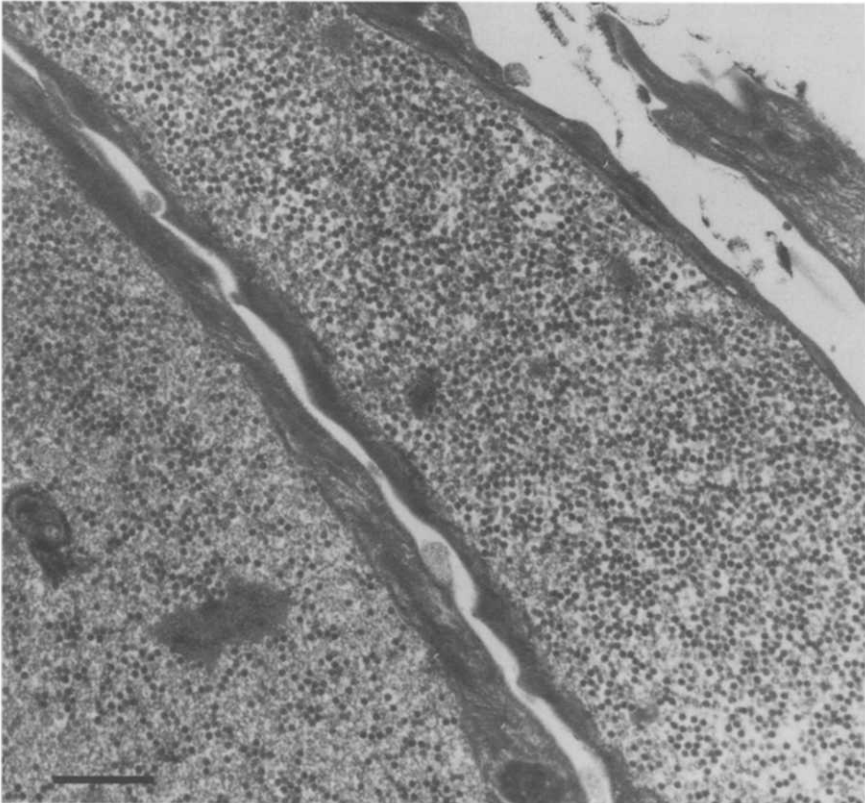


Fig. 4. Virus particles in the nuclei of two superficially located epidermal cells. Scale bar represents 500 nm.

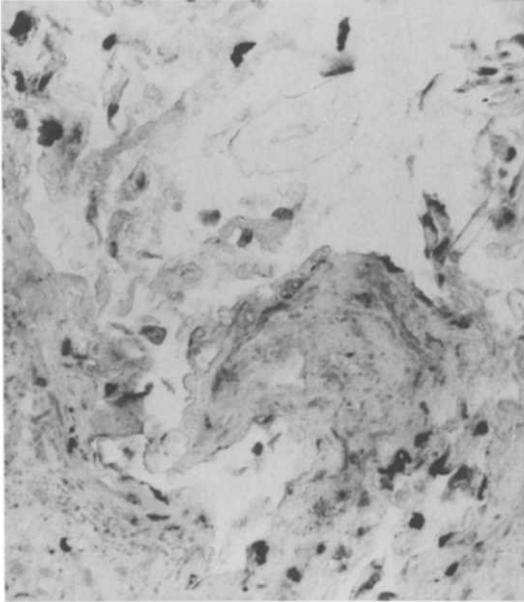


Fig. 5. Immunohistochemical staining of section through a papilloma-like lesion. The dark spots visible in this photograph represent the papillomavirus antigen. Reactions are seen mainly in the nuclei. APAAP; 400 \times .

talline aggregates were not detected. Based on their size, localization and general appearance, as well as on their presence in wart-like lesions the particles were provisionally classified as papillomavirions.

For a definite identification immunohistology was performed on sections through selected skin lesions. Staining of papillomavirus group-specific antigens was indeed obtained with the BPV-1 serum, mainly in the stratum granulosum and stratum corneum of the papilloma; some cells in the stratum spinosum also stained positive. Immunoreactivity appeared in the nuclei and to a lesser extent in the cytoplasm. No reaction was observed in the unaffected epithelium of the surrounding tissue. Sections mock-stained with normal rabbit serum were uniformly negative. Also in sections through the papillomatous lesions of 9 other cats no antigen was detected.

In view of the disease signs and the reported connection between papillomas and immune suppression in two cats (Carney et al., 1990), this cat's serum was tested for FIV antibodies by IFA. The positive diagnosis obtained was confirmed by western blotting: the serum reacted with the 3 FIV specific gag proteins. The cat tested negative for FeLV.

DISCUSSION

One year ago, the first report of papillomavirus infections in the cat was published in the USA (Carney et al., 1990). The morphology and cytopath-

ologic changes of the lesions reported here for a case identified in Europe are very similar to those described by Carney et al.. Here, too, the macroscopic appearance of the lesions was not typical of the papillomas seen in other domestic animals. Although the surface of the lesion was verrucous, they appeared as slightly raised plaques rather than as warts; also, they were pigmented. The cytopathologic changes showed the pattern characteristic for a papillomavirus infection. Although the hydropic degeneration of stratum spinosum cells seen in feline cowpox virus infections bears some resemblance, these lesions rather develop into reticular degeneration and microvesicles, resulting in the typical crater-shaped pocks. Moreover, in cowpox prominent viral inclusions are seen as acidophilic intracytoplasmic bodies (Gaskell et al., 1983).

There is no reported age resistance in papillomavirus infections; most lesions are found in young individuals but animals of all ages can be infected. The cat in our study was 6 years old. Human immunodeficiency virus (HIV), the cause of AIDS, has been identified as a factor of increased risk for clinical manifestations of papillomavirus infection in man (Henry et al., 1989; Matorras et al., 1991). The same appears to hold true for the feline condition: while the cats from the first report had been under immunosuppressive therapy (Carney et al., 1990), the animal in our study was infected with FIV. This infection is characterized by a latency period of several years after which time symptoms of secondary, opportunistic infections may develop (Yamamoto et al., 1988). As for HIV in man, FIV in cats causes a decrease of immune functions; decreased T-cell responses, low CD4 cell counts and inversed ratios of CD4:CD8 cells have been reported (Ackley et al., 1990; Siebelink et al., 1990; Barlough et al., 1991). Although immunological parameters were not determined in our case, the symptoms of opportunistic and chronic infections (otitis externa, *Microsporium canis* infection) that had developed a few months before appearance of the papillomas may be taken as an indication of immunodeficiency. Most FIV infected cats showing signs of disease are older than 5 years (Pedersen et al., 1989) which also applies to our case. The papillomas have persisted now for more than half a year; their regression may have been hampered because of the FIV infection.

The feline papovavirus described in the first report (Carney et al., 1990) was shown to be different from other papillomaviruses: the differences pertained to a unique staining pattern using a panel of monoclonal antibodies against papillomavirus capsid protein epitopes (Lim et al., 1990). The questions may be asked whether there really is an authentic feline papillomavirus and why it took so long before it was discovered. Transmission of papillomaviruses has so far only been reported within one animal species or between closely related hosts. Serological studies are required to determine the incidence of subclinical infections in the feline population. However unlikely, the epidemiology of papillomavirus infections may be similar to that of poxvi-

ruses, the cat serving as a dead-end host with occasional spill-over from another species, e.g. that of a prey animal. Another reason for the lack of reports on papillomas in cats certainly is the inconspicuous nature and uncharacteristic morphology of the lesions which have precluded a clinical diagnosis by analogy. We favour the explanation that papillomavirus infections occur regularly, their clinical manifestations being the consequence of acquired immune dysfunctions. Our observation and the cases reported previously (Carney et al., 1990) support this hypothesis.

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