The Journal of Veterinary Medical Science

NOTE

Pathology

Cutaneous papilloma and multicentric squamous cell carcinoma in four-toed hedgehogs (*Atelerix albiventris*)

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J. Vet. Med. Sci.
83(11): 1726–1729, 2021
doi: 10.1292/jvms.21-0302

Received: 27 May 2021
Accepted: 6 September 2021
Advanced Epub:
17 September 2021

ABSTRACT. Skin lesions possibly caused by Papillomavirus infections in two four-toed hedgehogs are described. In case 1, there was a papillary mass on the right hind limb. Histologically, the mass was consistent with a viral papilloma. In the other case, multifocal papillary masses with erosions and ulcers were found throughout the body, mainly on the extremities. Histology showed continuative lesions composed of acanthosis, Bowenoid *in situ* carcinoma, and squamous cell carcinoma, with abrupt transitions between the lesions. In both cases, keratinocytes in the granular layer infrequently had features of koilocytes and intranuclear inclusion bodies, and immunohistochemical staining was positive for anti-human papillomavirus antibody. To the best of the authors' knowledge, this is the first pathological documentation of possibly papillomavirus-associated skin lesions in four-toed hedgehogs.

KEY WORDS: four-toed hedgehog, histopathology, neoplasm, papillomavirus

Four-toed hedgehogs (*Atelerix albiventris*) are among the small mammals belonging to genus Atelerix and family Erinaceidae. Wild four-toed hedgehogs are native to the savannah and steppe regions of central and eastern Africa [4]. Four-toed hedgehogs are popular pets in the Americas, Europe, and some Asian countries including Japan. This species has been known to have a high incidence of neoplastic diseases, with a reported incidence of 53–60% [12, 13]. Various neoplasms have been reported; in particular, fibrosarcoma, mammary adenocarcinoma, and squamous carcinoma have been more commonly reported [12, 13]. Papillomavirus (PV)-associated skin lesions in four-toed hedgehogs have not been previously reported. The present report describes the clinical and pathological characteristics of skin lesions in two four-toed hedgehogs with a possible association with PV.

The animal 1 was a 2-year-and-9-month-old, male, four-toed hedgehog weighing 654 g. The animal was presented to the animal hospital with a chief complaint of recurrent skin lesions of both forelimbs and the right hind limb. Grossly, the left and right forelimbs had moderately elevated skin lesions with erosions and ulcers, and the surfaces were covered with crusts (Fig. 1a). These lesions measured 15 × 7 × 3 mm and 17 × 14 × 7 mm, respectively. There was a 5 × 4 × 2 mm, exophytic mass on the right hind limb (Fig. 1b). Skin biopsies were performed from both forelimbs and the mass of the right hindlimb. General bacterial culture detected *Enterococcus faecalis*, *Escherichia coli*, and *Proteus* sp. from the crusts of both forelimbs, with *Trichophyton* sp. detected by fungal culture. According to the results of antibacterial susceptibility tests, enrofloxacin (Reneval; Zoetis Japan, Tokyo, Japan), faropenem (Farom tablets; Maruho, Osaka, Japan), and itraconazole (Itraconazole tablets "Nichi-iko"; Nichi-Iko Pharmaceutical, Toyama, Japan) were prescribed for the infections. After the treatment, partial improvement of the lesions was obtained, but recurrence was seen seven months later with broader spread.

The animal 2 was 2-year-and-10-month-old, female, four-toed hedgehog weighing 310 g. The animal was presented to the animal hospital with a chief complaint of scaling, plaques, and pruritus of the extremities. On general bacterial culture, *Enterococcus casseliflavus*, *Enterococcus faecalis*, and *Escherichia coli* were detected. The animal was treated with selamectin (Revolution 6%; Zoetis Japan), enrofloxacin, and itraconazole for about two and a half months by the results of antibacterial susceptibility tests, with improvement of the lesions. Approximately 7 months later, similar skin lesions recurred and worsened,

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(Supplementary material: refer to PMC https://www.ncbi.nlm.nih.gov/pmc/journals/2350/)

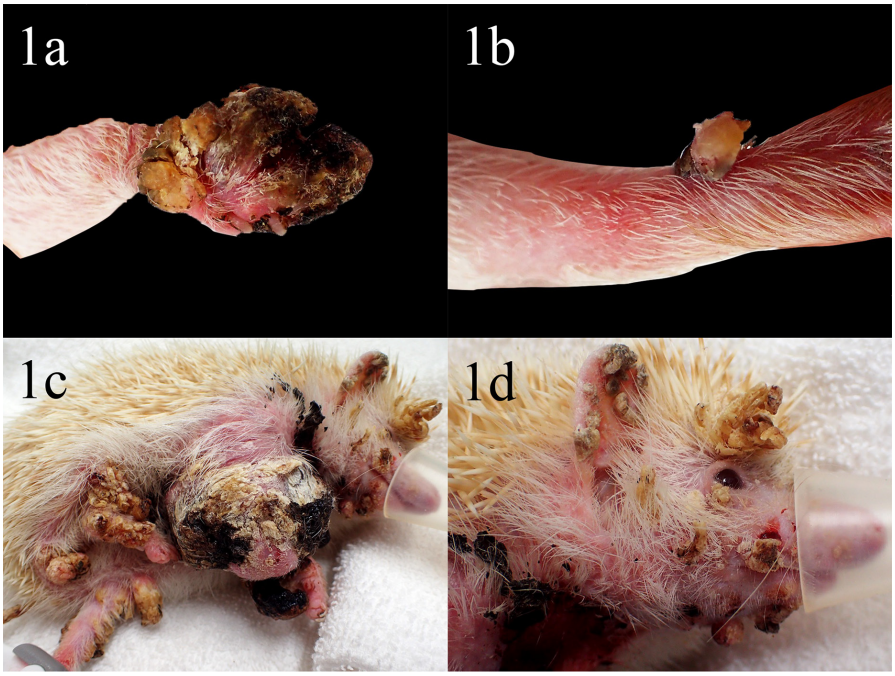

Fig. 1. Gross findings of the skin lesions in two four-toed hedgehogs. **(a)** Right forelimb of case 1. An elevated skin lesion with ulceration and crusts. **(b)** Right hind limb of case 1. A 5 × 4 × 2 mm, exophytic mass. **(c)** Whole body of case 2. Multifocally, plaques and raised nodular skin lesions were present throughout the body. A 30 × 20 × 15 mm, friable mass was present on the right forelimb. **(d)** Facial region of case 2. Skin lesions were frequently eroded and ulcerated, covered by crusts.

with spreading throughout the body (Fig. 1c and 1d). In addition, a 30 × 20 × 15 mm, raised, friable mass had formed from the right forelimb to the right axillary region. The surface of the mass was erosive and ulcerated. In other areas, irregular plaques with severe scaling were observed. Skin biopsies were performed from the right upper eyelid, submandibular region, ventral neck, left forelimb, right forelimb, left hind limb, right hind limb, and tail. These two animals have been kept by different owners.

In both cases, the skin samples were fixed in 10% neutral-buffered formalin and submitted to the Laboratory of Veterinary Pathology, Nihon University, for histopathological examination. After trimming, representative tissues were routinely processed, embedded in paraffin, sectioned at a thickness of 5 µm, and stained with hematoxylin and eosin. Immunohistochemistry was performed with routine methods using mouse monoclonal anti- PV antibody [cocktail of BPV-1/1H8 and CAMVIR] (diluted 1:160; Abcam, Cambridge, UK). This antibody reacts broadly to animal papillomaviruses including Canine, Bovine and Zalophus papillomaviruses [3]. After deparaffinisation, antigen retrieval was achieved using target retrieval solution 10 × concentrate (Dako North America, Carpinteria, CA, USA) at 121°C for 20 min. Labelling was visualized with 3,3-diaminobenzidine substrate (FUJIFILM Wako Pure Chemical Corp., Osaka, Japan), and sections were counterstained with Mayer's hematoxylin. Since there have been no previous reports of viral papilloma in the four-toed hedgehog, canine viral papilloma was used as control, and an appropriate reaction was confirmed. In addition, the DNA was extracted and purified from the formalin-fixed, paraffin-embedded (FFPE) samples of both cases using Gene Read DNA FFPE Kit (QIAGEN, Venlo, Netherlands), and nested and semi-nested polymerase chain reactions (PCR) were performed (NO, YI, SY and RK). The primer sets selected were My09/11 and MusPV-My09/11, as reported [7]. My09/11 is commonly used to detect many different types of PVs, and MusPV-My09/11 has been designed to specifically detect mouse PV. PCR was performed using a gradient of annealing temperatures from 50°C to 60°C and TakaRa Ex Taq (Takara Bio Inc., Kusatsu, Japan).

Histologically, the cutaneous mass on the right hind limb in case 1 consisted of epidermal thickening and exophytic growth due to keratinocyte proliferation, accompanied by prominent orthokeratotic hyperkeratosis (Fig. 2a). Keratinocytes were often enlarged, and some cells in the upper layers contained abundant, coarsely stippled, basophilic keratohyalin granules. No cellular and nuclear atypia was observed in these cells. Small numbers of keratinocytes in the superficial layer had pyknotic nuclei with intracytoplasmic perinuclear halos, showing koilocytes, and rare eosinophilic intranuclear inclusion bodies were noted (Fig. 2b). In the skin lesion on the left forelimb, there was marked orthokeratotic hyperkeratosis, and numerous fungal hyphae and bacterial rods were present between the keratin. In addition to the findings of the left forelimb, the skin from the right forelimb was covered with thick crusts composed of degenerate neutrophils, fibrin, and necrotic tissue. Based on these findings, the right hind limb was consistent with a viral papilloma, and the samples of both forelimbs were non-specific hyperkeratosis because of a lack of dermal tissue. In case 2, similar histological findings were observed in all samples, although the severity of the lesions varied. The epidermis was thickened by proliferation of keratinocytes accompanied by orthokeratotic hyperkeratosis. There were areas of papillary growth of the epidermis. Koilocytes and eosinophilic intranuclear inclusion bodies were infrequently noted. Multifocally,

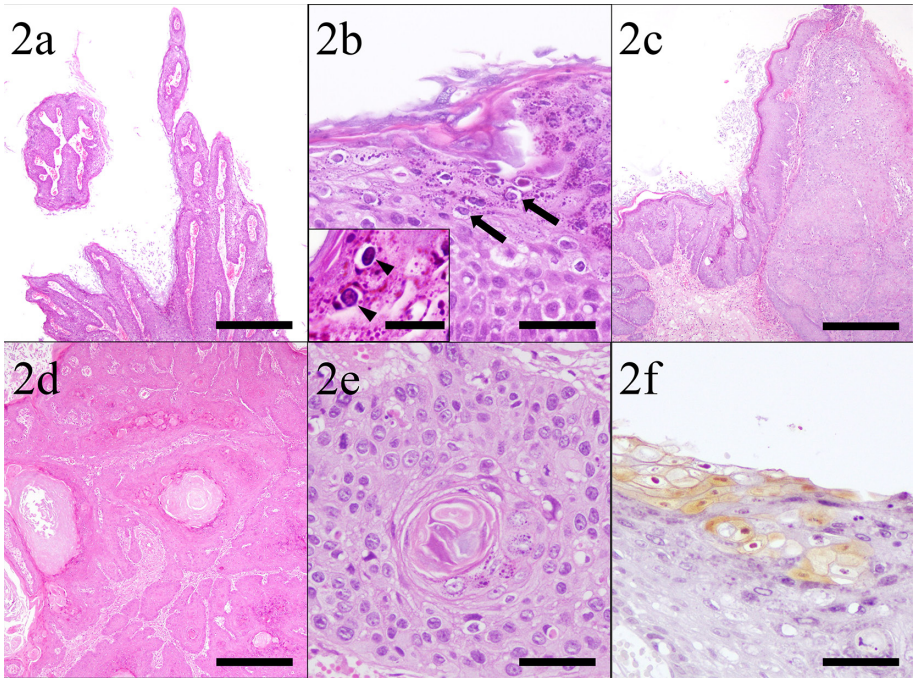

Fig. 2. Histological findings of the skin lesions in two four-toed hedgehogs. **(a)** Right hind limb of case 1. Papillary proliferation of keratinocytes with orthokeratotic hyperkeratosis was observed. Haematoxylin and eosin (H&E) stain. Bar=500 µm. **(b)** Right hind limb of case 1. In the superficial layer of the mass, koilocytes (black arrows) were occasionally observed. H&E stain. Bar=50 µm. Inset: Intranuclear eosinophilic inclusion bodies (black arrowheads) with margination of chromatin to the periphery were observed in the superficial part of the mass. Bar=20 µm. **(c)** Left hind limb in case 2. The continuous skin lesion composed of acanthosis (left side) and Bowenoid *in situ* carcinoma (right side). H&E stain. Bar=500 µm. **(d)** Left forelimb of case 2. Keratinocytes arranged in islands and trabeculae patterns infiltrated into the subjacent dermis. Bar=500 µm. **(e)** Right forelimb of case 2. Occasional keratin pearl formation was observed. H&E stain. Bar=50 µm. **(f)** Immunohistochemical staining of the mass on the right hind limb in case 1. In the granular to corneal layers, the nuclei of keratinocytes were positive for anti-PV antibody [cocktail of BPV-1/1H8 and CAMVIR]. Counterstained with Mayer's haematoxylin. Bar=50 µm.

there was an abrupt transition to the areas of marked epidermal thickening, with a loss of polarity of keratinocyte arrangement, although there was no invasion of the cells into the dermis (Fig. 2c). These cells showed moderate cellular and nuclear atypia, and mitoses were five per 10 high-power (400×) fields. Similar findings were observed in the adjacent follicular epithelium. In addition, areas of transition to squamous cell carcinoma were observed, and the lesions invaded the dermis (Fig. 2d). In these areas, neoplastic keratinocytes arranged in islands and trabeculae supported by moderate amounts of fibrous stroma were seen. Dyskeratotic cells and keratin pearl formation were frequently observed (Fig. 2e). Anisocytosis and anisokaryosis of the neoplastic cells were moderate, and mitoses were eight per 10 high-power (400×) fields. The lesions in case 2 were sequential lesions composed of acanthosis, Bowenoid *in situ* carcinoma (multicentric squamous cell carcinoma *in situ*), and squamous cell carcinoma.

Immunohistochemically, in case 1, nuclei of a small number of keratinocytes in the granular layer were positive for anti- PV antibody (Fig. 2f, Supplementary Figs. 1 and 2). In case 2, positive immunostaining was observed in areas of acanthosis and less frequently in areas of Bowenoid *in situ* carcinoma. No positivity was found in the areas of squamous cell carcinoma. No amplification of PV DNA was confirmed.

Both animals remain good physical condition 9 months and 5 months after the skin biopsies, respectively. However, skin lesions gradually worsened in both animals.

PV is a type of icosahedral double-stranded DNA virus [9]. With a few notable exceptions, PV is species-specific [14]. Although the role of PV in carcinogenesis has not been established in domestic animals, it is known to cause skin and mucosal lesions in many large animals [5]. Viral papillomas, especially in the skin, have been shown to be neoplasms caused by PV infection in a variety of animals [6]. PV infection is also associated with oral papilloma, inverted papilloma, and cutaneous viral pigmented plaque in dogs, and with viral plaque, Bowenoid *in situ* carcinoma, cutaneous squamous cell carcinoma, and sarcoid in cats [11]. In particular, Felis catus papillomavirus type-2 (Fca-PV2) is thought to be associated with a series of viral plaques, Bowenoid *in situ* carcinoma, and squamous cell carcinoma in cats [11]. Although cutaneous papillomatosis has been described in four-toed hedgehogs, no detailed histological findings suggesting viral involvement or viral infection have been described [15]. In the present study, case 1 was considered to be essentially the same lesion with a viral papilloma as observed in other animal species. Case 2 closely resembled a series of skin lesions caused by Fca-PV2 infection in cats.

Viral papillomas are benign proliferative epithelial tumors that occur in the epidermis due to infection with PV and form wart-like or filiform exophytic keratotic masses [6, 8]. The lesions may be extensive or multiple [8]. Histologically, there is acanthosis

and the presence of a small number of cells with grayish-blue cytoplasm in the spinous layer and cells with nuclei surrounded by distinct halos (koilocytes) [6, 11]. Keratohyalin granules may be aggregated in the granular layer, or eosinophilic intranuclear inclusions may be seen [11, 14]. The skin lesion in case 1 was consistent with a viral papilloma.

Viral plaque and Bowenoid *in situ* carcinoma are lesions caused by Fca-PV2 in cats [8]. Histologically, mild acanthosis and viral changes in the stratum spinosum are observed in viral plaque, including the presence of a small number of cells with grayish-blue cytoplasm and cells with nuclei surrounded by distinct halos (koilocytes) [8]. Bowenoid *in situ* carcinomas are multiple, crusted, and ulcerated plaques [9]. Histologically, there is thickening of the epidermis and atypia of keratinocytes, which occasionally extend into hair follicles, but no invasion into the dermis across the basement membrane [9]. These two diseases are known to be precancerous lesions that often progress to squamous cell carcinoma [2, 10]. In cats, PV DNA has been detected in some squamous cell carcinomas, and the E6 and E7 proteins produced by PV have been suggested to be related to neoplastic transformation [1, 10]. The lesions in case 2 were continuous lesions composed of a series of acanthosis, Bowenoid *in situ* carcinoma, and squamous cell carcinoma, each of which showed transition. In addition, small numbers of cells in the areas of acanthosis and Bowenoid *in situ* carcinoma showed positive immunostaining for anti-HPV antibody, suggesting that similar continuous skin lesions caused by PV in cats are present in four-toed hedgehogs.

Although the occurrence of cutaneous papillomatosis in four-toed hedgehogs has been described [15], the present study describes, for the first time, the clinical and histopathological findings of skin lesions suggestive of PV infection, evidence of viral antigens, and the presence of a series of PV-associated lesions reported especially in cats. However, PV DNA was not detected in the FFPE tissues from present cases. This result might be due to an inadequate quality of isolated DNA and specificity of primer sets used in PCR. Further studies are required to reveal the association between PV infection and skin lesions. For instance, changes in expressions of p53, p16, and retinoblastoma protein, as shown in a series of lesions in cats, were not evaluated because cross-reactivity of antibodies to hedgehog tissues has not been investigated. Our future plan includes investigation of the expression of these proteins in PV-associated skin lesions of four-toed hedgehogs, detection of viral DNA in the lesions, and DNA analysis to elucidate the pathogenesis of PV-associated lesions in this animal species.

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

REFERENCES

1. Altamura, G., Corteggio, A., Pacini, L., Conte, A., Pierantoni, G. M., Tommasino, M., Accardi, R. and Borzacchiello, G. 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. [Medline] [CrossRef]
2. Baer, K. E. and Helton, K. 1993. Multicentric squamous cell carcinoma in situ resembling Bowen's disease in cats. *Vet. Pathol.* **30**: 535–543. [Medline] [CrossRef]
3. Chang, C. Y., Chen, W. T., Haga, T., Yamashita, N., Lee, C. F., Tsuzuki, M. and Chang, H. W. 2020. The detection and association of canine papillomavirus with benign and malignant skin lesions in dogs. *Viruses* **12**: 170. [Medline] [CrossRef]
4. Doss, A. G. and Carpenter, J. W. 2020. African pygmy hedgehogs. pp. 401–415. In: Ferrets, Rabbits, and Rodents Clinical Medicine and Surgery, 4th ed. (Quesenberry, E. K., Orcutt, J. C., Mans, C. and Carpenter W. J. eds.), Elsevier, Amsterdam.
5. Gil da Costa, R. M., Peleteiro, M. C., Pires, M. A. and DiMaio, D. 2017. An update on canine, feline and bovine papillomaviruses. *Transbound. Emerg. Dis.* **64**: 1371–1379. [Medline] [CrossRef]
6. Goldschmidt, H. M. and Goldschmidt, H. K. 2017. Epithelial and melanocytic tumors of the skin. pp. 91–99. In: Tumors in Domestic Animals, 5th ed. (Meuten, D. J. ed.), Wiley Blackwell, New Jersey.
7. Joh, J., Jenson, A. B., Proctor, M., Ingle, A., Silva, K. A., Potter, C. S., Sundberg, J. P. and Ghim, S. J. 2012. Molecular diagnosis of a laboratory mouse papillomavirus (MusPV). *Exp. Mol. Pathol.* **93**: 416–421. [Medline] [CrossRef]
8. Mauldin, A. E. and Peters-Kennedy, J. 2015. Integumentary system. pp. 706–714. In: Pathology of Domestic Animals, Vol. 1, 6th ed. (Maxie, G. M. ed.), Elsevier, Amsterdam.
9. Munday, J. S. 2014. Papillomaviruses in felids. *Vet. J.* **199**: 340–347. [Medline] [CrossRef]
10. Munday, J. S., Sharp, C. R. and Beatty, J. A. 2019. Novel viruses: update on the significance of papillomavirus infections in cats. *J. Feline Med. Surg.* **21**: 409–418. [Medline] [CrossRef]
11. Munday, J. S., Thomson, N. A. and Luff, J. A. 2017. Papillomaviruses in dogs and cats. *Vet. J.* **225**: 23–31. [Medline] [CrossRef]
12. Okada, K., Kondo, H., Sumi, A. and Kagawa, Y. 2018. A retrospective study of disease incidence in African pygmy hedgehogs (*Atelerix albiventris*). *J. Vet. Med. Sci.* **80**: 1504–1510. [Medline] [CrossRef]
13. Raymond, J. T. and Garner, M. M. 2001. Spontaneous tumours in captive African hedgehogs (*Atelerix albiventris*): a retrospective study. *J. Comp. Pathol.* **124**: 128–133. [Medline] [CrossRef]
14. Sundberg, J. P., Van Ranst, M., Montali, R., Homer, B. L., Miller, W. H., Rowland, P. H., Scott, D. W., England, J. J., Dunstan, R. W., Mikaelian, I. and Jenson, A. B. 2000. Feline papillomas and papillomaviruses. *Vet. Pathol.* **37**: 1–10. [Medline] [CrossRef]
15. Turner, V. P., Brash, L. M. and Smith, A. D. 2018. Hedgehogs. pp. 387–416. In: Pathology of Small Mammal Pets (Turner, V. P., Brash, L. M. and Smith, A. D. eds.), Wiley Blackwell, New Jersey.