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Veterinary Dermatology

CASE REPORT

Detection of a novel papillomaviral sequence in viral plaques confined to the pinna of a dog

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Abstract

A raised plaque that contained histological evidence of papillomavirus infection and sequences from a novel papillomavirus type developed close to the ear canal of a 14-year-old West Highland white terrier. The plaque was excised, and further plaques developed within the same area of pinna.

KEYWORDS
aural plaques, canine papillomavirus, dog, papillomaviruses, pigmented plaques, viral oncogenesis, warts

INTRODUCTION

Plaques resulting from papillomavirus (PV) infection are uncommon cutaneous lesions of dogs. Canine cutaneous plaques typically appear as slightly raised pigmented lesions that are most common on the ventrum and medial surfaces of the limbs, although plaques can become more widespread over the body.[1] Unlike the commonly observed cutaneous or oral viral papillomas (warts) that are caused by PV types within either the *Lambdapapillomavirus* or *Taupapillomavirus* genera, plaques are caused by a number of closely related *Chipapillomavirus* PV types.[2,3] It is thought that immune dysfunction could play a significant role in lesion development by allowing increased PV replication.[1] Some breeds of dog, notably pugs, are predisposed to viral plaques, suggesting these breeds may have an inherited inability to mount an effective immune response against *Chipapillomavirus* infection.[4] The development of plaques also may be influenced by immunosuppressive diseases or treatments.[5] The present report describes plaques that were confined to one pinna of a dog. In addition to the unique location, the plaques also had unusual histological features and contained PV sequences from a putative novel *Chipapillomavirus* type.

CASE REPORT

A 14-year-old female spayed West Highland white terrier developed a small flat plaque close to the opening of the right external ear canal. The dog had a history of chronic otitis affecting this ear for the previous two years. Although the dog had undergone a bulla osteotomy approximately one year previously, she had continued to develop episodes of bacterial otitis which had been treated by flushing the ear and administering topical antimicrobials. The dog also previously had been diagnosed with allergic bronchitis and had been treated with 0.5 mg/kg prednisolone per os, once daily (Redipred; Aspen Pharmacare Australia) for approximately a year before presentation. An excisional biopsy of the lesion was taken for histological evaluation.

Histological results revealed a well-demarcated area of variable epidermal hyperplasia that resulted in multiple raised plaques of marked hyperplasia separated by narrow areas with more mild changes. The epidermis was not folded. Cells within basilar areas appeared crowded; however, the majority of the thickening resulted from expansion of the granular layer due to the presence of keratocytes that had large quantities of clear or faintly granular basophilic cytoplasm. These cells also contained large central nuclei that occasionally were darkened and surrounded by a clear

halo (koilocytes). While nucleoli often were prominent, intranuclear viral inclusions were not identified. The plaques were covered by a thick layer of parakeratosis. Increased lymphocytes and plasma cells were visible within the dermis underlying the plaque.

The lesion was diagnosed as a probable viral hyperplastic plaque although, as a consequence of the orientation of the sample within the histological block, an early squamous cell carcinoma (SCC) could not definitively be excluded. Recurrence was observed in the same location around two weeks after the first surgery with a larger (0.5 cm diameter) central mass surrounded by smaller, less raised, plaques. The large mass continued to grow and three months after the initial surgery, this lesion was 2 cm in diameter and raised 0.5 cm from the surrounding skin of the pinna. The masses remained confined to the skin surrounding the opening of the external ear canal (Figure 1) and no additional plaques were visible elsewhere on the body. Owing to the possibility that the lesion could be an SCC, an additional sample was taken. Histological results revealed similar lesions as before (Figure 2), although clear visualisation of the basement membrane allowed exclusion of an SCC.

As a consequence of the histological evidence of PV infection, total DNA was extracted from formalin-fixed, paraffin-embedded samples of both excised plaques and the MY09/11 and CP4/5 consensus PCR primers were used to amplify PV DNA.[6] Both primer sets amplified PV DNA from both plaques. When the DNA that had been amplified by the MY09/11 primers was sequenced, the PV DNA sequences were identical from the initial plaque and the recurrent lesion. This sequence was compared to other sequences in the GenBank database using the BLAST tool. This revealed that the 271 bp sequence amplified from both plaques had the greatest (85.9%) similarity to CPV15, and second highest similarity to CPV8 (71.9%). The novel sequence was deposited in GenBank under accession number OP645388.

The plaques continued to grow slowly in the four months since the last sample was taken, and, although the large central mass was around 3 cm in diameter at the time of writing, the masses did not appear to cause irritation to the dog.

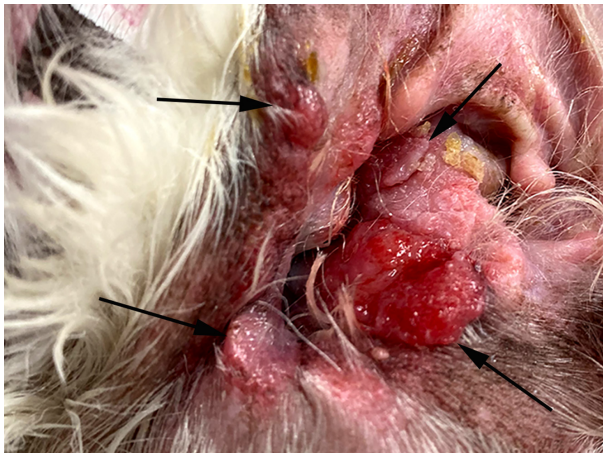

FIGURE 1 Cutaneous viral plaques. The plaques appear as multiple raised lesions on the inside surface of the pinna (arrows). Plaques were restricted to the skin adjacent to the opening of the external ear canal.

DISCUSSION

The cutaneous plaques in the present case contained a DNA sequence from a novel PV type. Detection of a PV sequence in a lesion does not confirm that the PV caused the lesion. However, the plaques contained histological evidence supporting a PV aetiology. Additionally, the same novel PV DNA sequence was detected in samples of both the initial and the recurrent plaques. Furthermore, no other PV type was amplified from the plaques in this case. Therefore, while additional cases are required, the putative novel PV type appears likely to be the cause of the plaques described herein. Definitive classification of a new PV type is only possible when the complete L1 sequence is known[7]; however, the putative novel PV type amplified from the plaques appears most likely to be within the *Chipapillomavirus* genus with the sequence much less similar to PV types of other PV genera.

Cutaneous viral plaques are well-recognised in dogs, the present case is unique as plaques have not previously been reported to be confined to the ear of a dog. Why the plaques developed at this location cannot be determined. However, the chipapillomaviruses are thought to cause viral plaques as a result of the immune system being unable to inhibit viral replication.[1] In the present case, the dog had otitis which resulted in chronic discharge from the affected ear. This discharge, along with the probable self-trauma to the ear, could have damaged the skin in this area, and may therefore have reduced the normal skin defences against PV infection. This reduced skin defence then could have allowed more rapid PV replication, resulting in the development of a visible plaque. Supporting a link between otitis and plaque development in this dog was the restriction of plaques to the skin surrounding the opening of the affected ear canal. However, to the best of the authors' knowledge, areas of chronic inflammation have not previously been reported to be predisposed to plaque formation in dogs or other species.

In humans, the development of cutaneous plaques is a well-recognised risk of immunosuppressive medications such as those used after organ transplantation.[8] Likewise in dogs, there are rare reports of plaques developing in association with immunosuppressive treatments or diseases.[5] Therefore, in addition to the local skin inflammation, the development of the plaques in the present case also could have been influenced by the chronic administration of low doses of corticosteroids. However, as many dogs are treated with corticosteroids without developing PV-induced lesions, the significance of the corticosteroids in the present case is uncertain.

The lesions in the present case were classified as plaques rather than warts owing to the clinical presentation, histological appearance of the lesions and

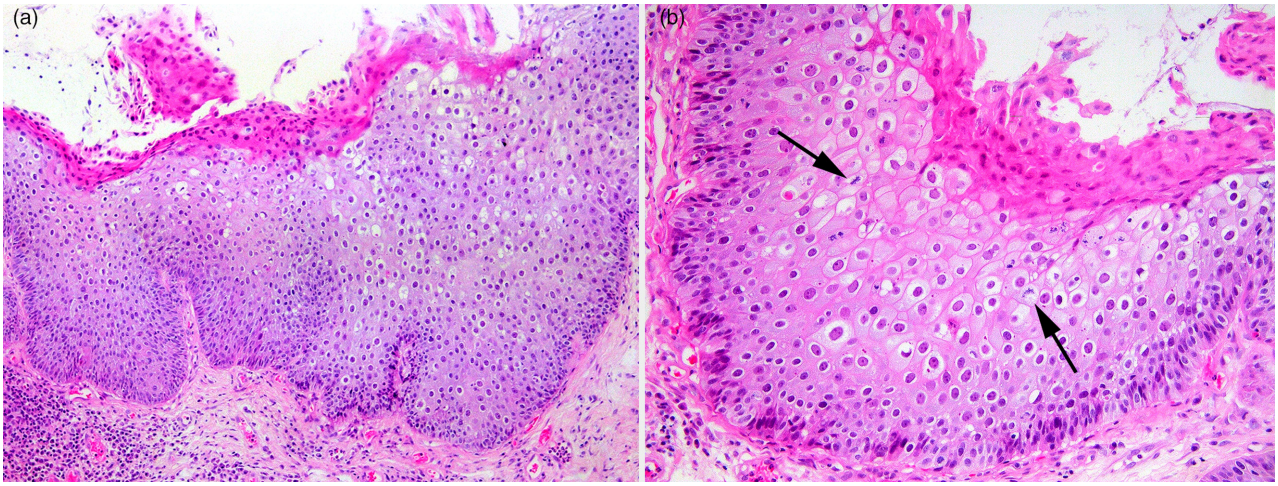

FIGURE 2 Cutaneous viral plaque, histological results. (a) The plaque consists of irregularly thickened epidermis covered by parakeratosis. Expansion of the epidermis is most marked within the stratum spinosum with keratinocytes enlarged by increased cytoplasm. (b) Keratinocytes within the plaques have increased qualities of vacuolated or granular grey-blue or clear cytoplasm. Cell nuclei are enlarged, central and prominent. Some cells contain darkened nuclei surrounded by a clear halo (koilocytes). Mitotic figures are visible within the hyperplastic epidermis (arrows).

the presumptive aetiology.[9] The clinical features supporting a viral plaque rather than wart included the recurrence after excision, the slow progression of the lesions and the failure to spontaneous resolve.[10] Additionally, the lesions were only mildly exophytic in the present case rather than being filiform or papillary as is more often seen with warts. Histologically, the lesions were formed as a result of moderate epidermal expansion. This is in contrast to the massive epidermal thickening and folding typically present in a viral wart.[11] However, the presently described lesions did not contain pigment within the underlying dermis or the typical 'scalloped appearance' that usually is present within a cutaneous viral plaque.[11] Additionally, the aural lesions contained marked PV-induced cytological changes. Such changes are to the result of PV replication in the lesion and are observed only rarely in cutaneous viral plaques in dogs, although they typically are visible in warts.[3] Finally, a diagnosis of viral plaque was supported by the detection of a putative *Chipapillomavirus* PV type within the lesion. Viral plaques are associated with *Chipapillomavirus* PV types while warts are caused by PV types within the *Lambdapapillomavirus* or *Taupapillomavirus* genera.[9]

Although 13 different *Chipapillomavirus* types have been detected in previous studies of canine viral plaques,[12] the putative novel PV type amplified from the pinnal plaques has not been reported previously. This could suggest that the novel PV type is a very uncommon cause of viral plaques in dogs. Alternatively, some PV types have been shown to have a strong tropism for specific areas of the body.[13] As this is the first report of plaques being confined to the pinna of a dog, it is possible that the novel PV type may preferentially infect skin in this area and so be more likely to cause plaques close to the ear than elsewhere on the body. Overall, the results from this case add to the clinical manifestations of PV-induced skin disease of dogs, as well as adding to the number of different PV types that can cause disease in dogs.

AUTHOR CONTRIBUTIONS

John S. Munday: Conceptualisation; writing – original draft; methodology. **Geoff Orbell:** Investigation; writing – review & editing. **Lynne Robinson:** Investigation; writing – review & editing.

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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Résumé
Une plaque virale à papillomavirus confirmée histologiquement contenant des séquences d'un un nouveau type de papillomavirus se sont développées à proximité du conduit auditif d'un West Highland White âgé de 14 ans. La plaque a été retirée chirurgicalement et d'autres plaques se sont développées dans la même zone du pavillon.

Resumen
Una placa elevada que contenía evidencia histológica de infección por papilomavirus y secuencias de un nuevo tipo de papilomavirus se desarrolló cerca del canal auditivo de un West Highland White Terrier de 14 años. Se extirpó la placa y se desarrollaron más placas dentro de la misma área del pabellón auricular.

Zusammenfassung
Nahe dem Ohrkanal eines 14-jährigen West Highland White Terriers entwickelte sich eine erhabene Plaque, die histologisch auf eine Papillomavirus Infektion hinwies und Sequenzen eines neuen Papillomavirus Typs zeigte. Diese Plaque wurde chirurgisch entfernt und es entwickelten sich weitere Plaques innerhalb derselben Stellen der Pinna.

要約
14歳のウエスト・ハイランド・ホワイト・テリアの外耳道付近に、パピローマウイルス感染の組織学的証拠および新規パピローマウイルス型の配列を含む隆起性局面が発生した。この局面を切除したところ、耳介の同部位にさらに局面が発生した。

摘要
在一只14岁的西高地白㹴犬耳道附近发现了一个隆起斑块，组织学证据含有乳头瘤病毒感染，而且是一种新型乳头瘤病毒序列。斑块被切除后，在耳廓的同一区域内出现更多斑块。

Resumo
Uma placa elevada apresentando evidências histopatológicas de infecção por papilomavírus e sequências de um novo tipo de papilomavírus surgiu próximo ao conduto auditivo de um West Highland White Terrier de 14 anos de idade. A placa foi removida e outras placas se desenvolveram na mesma área da orelha.