Papillomaviruses (PVs) can infect epithelia and induce proliferative disorders. Different types of canine PVs have been found to be associated with distinct pathologies including exophytic warts as in canine oral papillomatosis, endophytic warts, and pigmented plaques and, in some cases, squamous cell carcinomas.

**ETIOLOGY**

PVs are double-stranded DNA viruses with a circular genome of about 8000 base pairs that is contained in a nonenveloped 50- to 55-nm icosahedral capsid. The capsid consists of the 2 structural proteins, L1 and L2, at a ratio of 30:1 and exposes primarily pentamers of L1 to the outside.\(^1\)\(^–\)\(^3\) All genetic information in the PV genome is encoded on the same DNA strand, and usually 6 to 8 open reading frames (ORFs) can be identified.\(^4\) Those ORFs are the late ones, L1 and L2, and the early ones, E1, E2, E4, E6, and E7. While L1, L2, E1, and E2 are present in all PVs, E4 is not always easily identified, as it is contained within the E2 ORF. E6 and E7 are present in most but not all known PVs. Some, like the members of the genus *Alpha-Papillomaviruses*, also contain an E5 ORF.\(^5\)

Originally, PVs had been allocated to the family Papovaviridae together with the polyomaviruses because of similarities in genome and capsid structure.\(^3\) As genome size and organization were found to differ and no significant sequence homologies could be identified, this categorization was abandoned and the taxonomic families Polyomaviridae and Papillomaviridae were established. The current classification of PVs within the Papillomaviridae family is based on nucleotide sequence identities of the L1 ORF. The categories genus (<60% identities), species (more than 60% identities), type (more than 70% identities), subtype (more than 90% identities), and variant (more than 98% identities) were introduced for further description of PV isolates.\(^5\) To date 29 PV genera have been recognized containing almost 200 distinct
PV types. The number of known PVs is growing constantly, but so far more than half of the published sequences derive from PVs infecting the human host.

PVs are species-specific pathogens, and the target tissues of most of them are keratinizing and mucous membranes. However, there are exceptions. The bovine PVs BPV1 and BPV2 naturally infect the cow as well as the horse and can experimentally also infect rodents. It also appears that the PV involved in feline sarcoids is of bovine origin. PVs induce a broad spectrum of benign epithelial neoplasias but are also known to be involved in the development of malignant neoplasias or are suspected to be.

PV infections in dogs have repeatedly been described since the late 19th century, and thus far the entire sequences of 7 canine PVs (CPVs) have been published as well as several short stretches of other putative CPVs. The 7 genomes of CPVs have been allocated to 3 different PV genera: Lambda, Tau, and Chi (Table 1). While Lambda contains also PVs of other carnivore species, Tau and Chi contain only CPVs so far.

### EPIDEMIOLOGY

The primary target cells of infectious PV particles are epithelial cells. Not all epithelial cells are, however, capable of cell division, which is mandatory for PVs to establish persisting infections. In case of the squamous epithelium, such cells are only found in the basal cell layer, protected by several layers of differentiated and differentiating keratinocytes. It was concluded that PVs require sites of injuries to be able to make contact with those basal cells. The virus seems to enter the cell mainly by clathrin-dependent receptor-mediated endocytosis after binding to integrin. During the following first phase of a PV infection, there is an initial amplification of the viral DNA in the nucleus, whereas thereafter it is copied about once per cell cycle in synchrony with the host genome. This phase is also characterized by a lack of clinical symptoms. It has been demonstrated, that in case of experimental infections, this phase lasts at least 4 weeks before the onset of apparent symptoms. Not all natural infections seem to involve the development of overt symptoms, though, and viral DNA can be detected on the clinically health skin of humans and several animal species.

Dogs have as well been shown to harbor the DNA of PVs in the absence of clinical symptoms to a high proportion (more than 50%) on their skin. It is, however, not clear whether these are cases of subclinical infections or if dogs just carry PVs or their DNA on their skin. A study on the prevalence of antibodies against CPV1 and CPV3 indicates that, depending on the population and the cut-off value chosen, up to about 50% may have had contact with at least 1 of these 2 PVs.

### Table 1

Canine papillomaviruses and clinical symptoms

<table>
<thead>
<tr>
<th>Virus</th>
<th>PV Genus</th>
<th>Described Clinical Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPV1</td>
<td>Lambda</td>
<td>Asymptomatic infections, exophytic papillomas, endophytic papillomas, invasive SCCs</td>
</tr>
<tr>
<td>CPV2</td>
<td>Tau</td>
<td>Exophytic papillomas, endophytic papillomas, invasive SCCs</td>
</tr>
<tr>
<td>CPV3</td>
<td>Chi</td>
<td>Pigmented plaques, in situ SCC, invasive SCC</td>
</tr>
<tr>
<td>CPV4</td>
<td>Chi</td>
<td>Pigmented plaques</td>
</tr>
<tr>
<td>CPV5</td>
<td>Chi</td>
<td>Pigmented plaques</td>
</tr>
<tr>
<td>CPV6</td>
<td>Lambda</td>
<td>Endophytic papillomas</td>
</tr>
<tr>
<td>CPV7</td>
<td>Tau</td>
<td>Exophytic papillomas, in situ SCC</td>
</tr>
</tbody>
</table>
The immune system of the host plays an important role in the outcome of PV infections, where the cellular immunity is mainly responsible for virus eradication while the humoral immunity protects the organism against reinfections. This is probably true for PV infections in all species. Thus, PV infections pose a greater threat for immunocompromised than for immunocompetent animals. This was supported by several reports, especially by the observation of a severe outbreak of CPV2-associated papillomatosis in a population of dogs with severe combined immunodeficiency (SCID). Also, reports of corticosteroid- or cyclosporine A–induced cases of papillomatosis support the role of the immune response in the dog. There are also indications that breed predispositions putatively associated with an inherited immune defect may exist, although the available data are very limited. The pug, as perhaps the best-documented example, seems to be prone to develop pigmented plaques.

PATHOGENESIS

The whole PV life cycle is closely linked to its host cells and thus to the differentiation program of the squamous epithelium. Most of the early genes are expressed primarily in the basal and suprabasal levels of the epithelium, while the late genes are exclusively expressed in the spinous and granular cell layers. Consequently, the assembly of virions occurs in the upper stratum granulosum and stratum corneum. The infective viruses are probably released due to normal death of cells in these layers, as PVs are not lytic viruses. In case of canine oral papillomatosis, hyperplasia develops in the stratum spinosum after the initial phase of subclinical infection, and wart formation with acanthosis and hyperkeratosis occurs. Most cytopathic effects such as intracytoplasmic pseudoinclusions, koilocytosis, and clumped keratohyalin granules can primarily be observed in the mid and upper epidermis, while intranuclear inclusions are only present in the upper epidermis. Spontaneous wart regression at 4 to 8 weeks after the onset of symptoms is part of the common course of PV infections inducing exophytic warts like oral papillomatosis. However, age and immune status of the dog determine the outcome of such infections. While in young dogs transient infections seem to be the rule, older and/or immunosuppressed dogs not only have a higher risk of developing clinical disease but also suffer from persistent infection and neoplastic transformation.

The outcome of PV infections probably depends on factors in addition to host immunity and genetic background. Among those influential factors may be the intrinsic pathogenicity of the involved PV type, subtype, or variant, as well as putative external factors. While the connection between PVs and benign neoplasias, primarily the canine oral papillomatosis, is well established, making a direct causal link between CPV infection and the development of malignant neoplastic transformation is rather difficult. Although there is some epidemiologic evidence for such a correlation, definite proof from in vitro experiments is missing. The individual differences in the outcome of PV infections can be illustrated based on an described outbreak of papillomatosis in a group of X-linked SCID dogs. Although all individuals were of the same breed, similarly immunocompromised and infected by the same pathogen (CPV2), the range of diagnosed lesions included exophytic and endophytic papillomas as well as, in some cases, in situ and even invasive squamous cell carcinomas (SCCs). In case of CPV3, which was isolated from a Rhodesian ridgeback, pigmented plaques, in situ and invasive SCC were found alongside in the same dog putatively marking different stages of PV infection. Although a serologic study indicated the prevalence of the virus, no further cases associated with this virus have been described yet. Even the mechanisms involved in the
relatively well-studied CPV1 infections that are responsible for canine oral papillomatosis seem to be understood only partially.

**CLINICAL FINDINGS**

Dogs may display a variety of CPV-associated skin disorders including classic warts with exophytic or endophytic growth, pigmented plaques, hyperkeratotic to horny lesions, and, in some cases, in situ or invasive SCCs (Table 1).13,14,16,19,20,22,39,43,50,52–65

**Canine Oral Papillomatosis**

Mainly young dogs are affected by canine oral papillomatosis, which appears in a broad spectrum of forms in the oral cavity but is not restricted to it.15,30 It is typically characterized by cauliflower like exophytic warts, but the benign tumors may as well be fringed or nodular (Fig. 1). The mainly affected tissue is the oral mucosa including the lips and mucocutaneous junctions. Tongue and esophagus are only occasionally afflicted. In some cases, the eyelids are also affected and papillomas infrequently occur on the haired skin in this context. Often these papillomas come in small numbers, but occasionally severe manifestations of oral papillomatoses are seen (Fig. 2). In larger dog colonies, outbreaks affect a varying proportion of animals.66 The virus involved in this oral papillomatosis complex was originally named canine oral papillomavirus (COPV), but other names were also sometimes used. In order to avoid confusion and for more uniformity in PV nomenclature, it was recently suggested to use the term *canine papillomavirus 1* (CPV1).6 Although CPV1 is primarily involved in the transient oral papillomatosis, it has also been reported to be possibly involved in nonregressing lesions and the development of SCCs and endophytic papillomas.21,50,67 Oral papillomatosis has also been observed in the coyote (Canis latrans) and the wolf (Canis lupus). As they are closely related to the domestic dog, it can be expected that the same virus is involved in the development of the lesions.68–72

**Inverted Papillomas**

Inverted papillomas or endophytic warts are characterized by a growth downward into the skin. This development results in raised and smooth nodules with a central pore filled with keratin. In histology endophytic, papillary projections of the epidermis
extending into the dermis are typical. Cytopathic effects are usually present in the form of clumped keratohyaline granules, koilocytes, and, less frequently, basophilic or eosinophilic inclusions. Four different subtypes have been described in dogs. Classic inverted papillomas, which were initially described by Campbell et al,\textsuperscript{58} have a diameter of 1- to 2-cm and are rather large, cup-shaped, grayish nodules with a large central pore (Fig. 3). They are typically found at the abdomen in small numbers or as solitary lesions. Shimada et al\textsuperscript{61} described a second type of inverted papilloma where the lesions have a diameter of about 4 mm. They present as dome-shaped flesh-colored papillomas, which may be disseminated over the whole body. A third type described by Le Net et al\textsuperscript{39} is characterized by even smaller (2 mm) disseminated black papules that display intracytoplasmatic eosinoplyic inclusions on histopathologic assessment. A fourth type described by Goldschmidt et al,\textsuperscript{13} that mainly resembles classic inverted papillomas, seems to be prone to induce interdigital lesions. Distinct PV types have been isolated from each of these lesions, but except for perhaps CPV2-associated papillomas, data are limited.\textsuperscript{13,18,21}

**Pigmented Plaques**

Canine pigmented plaques consist of small (1 mm) to medium-sized (1 cm), dark, plaquelike hyperkeratotic lesions that predominantly show up at the limbs, axillae, or
abdomen (Fig. 4). They are usually very flat but may be slightly raised and usually appear in clusters. Pigmented plaques were initially referred to as lentiginosis profuse.\textsuperscript{42} However, as the association between CPV and this condition was demonstrated, it was hypothesized that it could be the canine counterpart of human epidermodysplasia verruciformis.\textsuperscript{73} Some major differences exist, though, between these conditions, and any premature comparison should consequently be avoided.\textsuperscript{48}

In contrast to typical exophytic or endophytic papillomas, canine pigmented plaques show little tendency for spontaneous regression. In pugs, this condition has repeatedly been reported, and the DNA of CPV4 was connected to it in all tested cases. Progression into malignant lesions was not reported in the pug.\textsuperscript{19,73–75} In the case of CPV3 infection and in the case of at least one other uncharacterized PV, a causal relation between virus, pigmented plaques, and in situ and invasive SCC seems to be evident (Fig. 5).\textsuperscript{14,22,51} CPV5 was also discovered in a dog with pigmented plaques but no signs of cancerous transformation were noticed. All viruses thus far connected with pigmented lesions are or seem to belong to the PV genus Chi.\textsuperscript{14,19,20,22}

**DIAGNOSIS**

The diagnosis of CPV-associated disorders depends on the type of disorder but may in general be based on gross appearance of the lesions and the epidemiologic
background. The methods most frequently used are classic histopathology and polymerase chain reaction (PCR).

Canine oral papillomatosis has a very obvious clinical presentation and may therefore be diagnosed without any laboratory testing, when observed in young dogs. Because other cases of suspected PV-induced papillomatosis are not as distinct in terms of morphology and epidemiology, full-thickness excision biopsy samples of entire lesions including some adjacent normal tissue should be obtained to perform histologic examination.

Classic warts usually reveal hyperplasia of the epidermis with extensive orthokeratotic hyperkeratosis. Typical features are clumped keratohyalin granules in the stratum spinosum, koilocytes (keratinocytes with swollen, clear cytoplasm and a pyknotic nucleus), clear cells (keratinocytes with swollen, blue-gray cytoplasm and enlarged nuclei), and intranuclear inclusion bodies (Fig. 6). In endophytic papillomas, centripetal papillary projections of hyperplastic squamous epithelium with a central core of keratin layers and parakeratotic cells are typically observed. The stratum spinosum is usually found to show irregular hyperplasia and contains moderate numbers of mitotic figures and dysplastic cells. The subcorneal epithelium displays a variable number of koilocytes, which may have basophilic intranuclear inclusions and a few large keratohyalin granules. In the case of the subtype described by Le Net et al, however, large eosinophilic intranuclear inclusions are apparent.

In pigmented plaques, moderate acanthosis with scalloped configuration, hyperpigmentation, and clumped keratohyalin granules in the stratum spinosum are typically found, while koilocytes as well as viral inclusions are usually not observed (Fig. 7).

PCR assays have been established for the detection of CPV DNA and can be applied to test material from biopsy or cytobrush samples. The assays are very sensitive, and short stretches of the viral genome may be determined using direct sequencing. However, as PV DNA may be found independent of clinical symptoms, PCR results have to be interpreted with caution and should be correlated with histopathology, clinical lesions, and epidemiologic data if available.

Additional methods that can be very helpful in verifying a diagnosis of CPV-associated papillomatosis are immunohistochemistry, in situ hybridization, electron
microscopy, and rolling circle amplification. Immunohistochemistry is a very informative method. It requires a decent amount of viral protein to provide a signal, but when positive, it clearly proves viral activity. However, no CPV-specific antibodies are commercially available. Antibodies against conserved regions of PV proteins have been used in the past. In situ hybridization can be used to locate nucleic acids of the virus in fixed tissues, thus determining the infected tissues and cells, and to show viral transcription when targeting RNA. Electron microscopy can be used to actually visualize characteristic viral structures to prove productive infection. Finally, rolling circle amplification is a method to amplify whole viral genomes independent of the sequence, which can be applied to fresh, but not fixed, samples. It enables the detection and characterization of already known as well as of unknown PVs as long as the circular form of PV DNA is not dissolved.

TREATMENT AND PREVENTION

The transient character of canine oral papillomatosis was already demonstrated more than 100 years ago. As transience is probably a feature of most PV-induced lesions, putative therapeutic approaches for papillomas should be reviewed with caution. Spontaneous regression and therapeutic effect are, under these circumstances, hard to discriminate, and the statement that “the credit claimed for some methods of treatment may be undeserved” remains relevant. Most papillomas will spontaneously regress within 1 to 2 months. The treatment of choice for papillomas that do not regress and cause severe problems due to their size or location is surgery. It should nevertheless be kept in mind that surgical excision has been reported to be associated with latent infection and increased recurrence. Medical treatments with interferons as well as with the immune modulator imiquimod have been suggested, but so far no studies on their effectiveness in canine papillomatosis have been published.

It was shown in 1898 and repeatedly confirmed that dogs that had suffered from oral papillomatosis are apparently protected from reinfections. The use of inactivated crude wart extract as a prophylactic vaccine had been demonstrated to be effective in preventing oral papillomatosis, and such techniques have repeatedly been used to protect larger dog populations from clinical papillomatosis. More recent approaches to develop effective and save vaccines in the lab are very promising. Viruslike particles consisting exclusively of CPV1 L1 capsid
protein could be produced. It was also possible to induce cellular and humoral immunity by the administration of plasmids coding for a few genes or a single gene of CPV1. While preventative vaccines against some human PVs involved in the development of cervix carcinomas are now on the market, commercial vaccines against CPVs are not available.

SUMMARY

PVs can infect epithelia and induce proliferative disorders. Different types of CPVs have been found to be associated with distinct pathologies including exophytic warts as in canine oral papillomatosis, endophytic warts, and pigmented plaques and, in some cases, squamous cell carcinomas. Virus infection is followed by a phase of subclinical infection before the onset of symptoms. A diagnosis can in some cases be made clinically but should be verified if there are any doubts. Most papillomas do regress spontaneously within a few months. Preventative vaccination is possible but not on the market.

REFERENCES


