Detection of canine oral papillomavirus (COPV) in conjunctival plaque and papillomas in three dogs

Kristin Brandes[1], J. Fritsche[2], N. Mueller[3], B. Koerschgen[3], B. Dierig[4], G. Strebelow[5], and J. P. Teifke[5]

[1]Animal Pathology Augsburg, Germany, [2]Veterinarian Practice for Ophthalmology, Munich, Germany,
[3]Veterinary Eye Specialists, Pohlheim, Germany, [4]Veterinary Practice, Neusaess, Germany,
[5]Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Isle of Riems, Germany

Introduction:
Papillomavirus infections are responsible for epithelial plaques and papillomas in various locations on the skin and in mucous membranes. Viral conjunctival papilloma of the third eyelid or conjunctiva is not yet included in the proposed WHO histological classification of ocular tumors of domestic animals. The aim of this presentation is to describe distinct and different morphological features of a viral pigmented conjunctival plaque and two conjunctival squamous papillomas in three dogs, and to investigate these lesions for the presence of papillomavirus DNA by means of polymerase chain reaction (PCR), DNA sequence analysis and *in situ* hydridization (ISH).

Material and methods:
Dog No.1: a 2.5-year-old intact male Cavalier King Charles Spaniel was presented with a pigmented circular elevated plaque on the limbal conjunctiva (Fig. 1). Dog No. 2: a 2-year-old intact male Jack Russell Terrier mongrel showed a raised neoplasm with verrucous fronds on the limbal conjunctiva (Fig. 2). Dog No. 3: a 2-year-old female Newfoundland dog, was presented with a wart on the conjunctiva of the third eyelid, 0.7 cm in diameter (Fig. 3). The entire conjunctival neoplastic tissue was removed, fixed in 4 % formaldehyde, routinely processed, embedded in paraffin wax, cut at 3 µm and stained hematoxylin-eosin (HE). To investigate the paraffin-embedded material for the presence of papillomavirus antigens, PCR, DNA sequence analysis and ISH were applied.

Teifke JP et al.: Detection of canine oral papillomavirus-DNA in canine oral squamous cell carcinoma and p53 overexpressing skin papillomas of the dog using the polymerase chain reaction and non-radioactive in situ hybridization. Vet Microbiol 60:119-130, 1998.

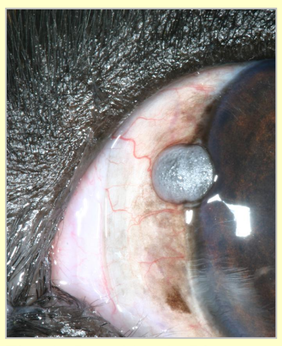

Fig. 1: Dog No. 1. Pigmented plaque on the limbal conjunctiva.

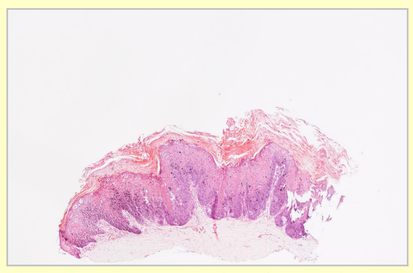

Fig. 1.1: Dog No.1. The plaque shows epithelial hyperplasia, acanthosis and marked hyperkeratosis, HE, 40x.

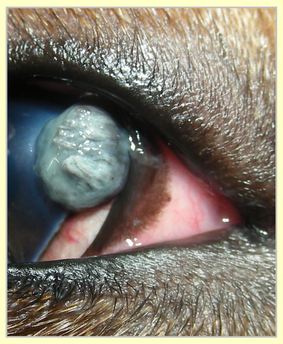

Fig. 2: Dog No. 2. Exophytic papilloma on limbal conjunctiva.

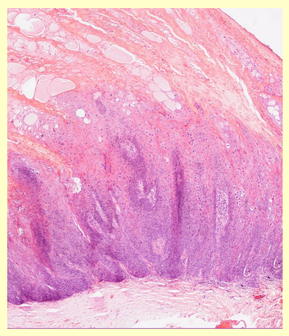

Fig. 2.1: Dog No. 2. Papilloma with numerous papillary projections, HE, 40x.

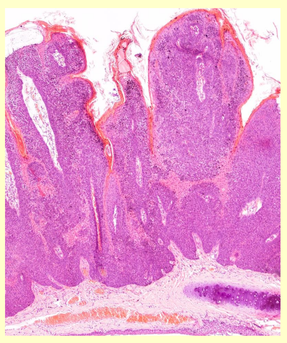

Fig. 3: Dog No. 3. Papilloma on the third eyelid. Note cartilage on the base, HE, 40x .

Viral cytopathic effects in all three neoplasms

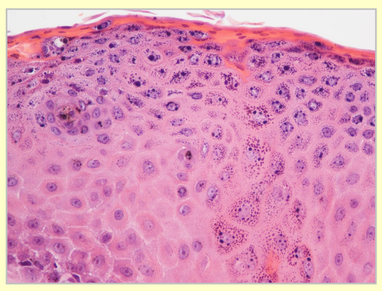

Fig. 4: Papilloma. Increase in number and size of keratohyalin granules (hypergranulosis), HE, 400x.

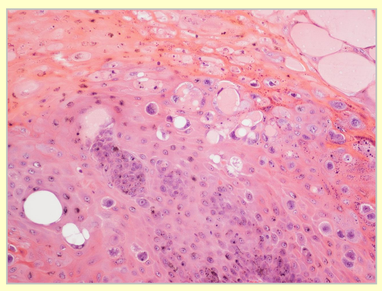

Fig. 5: Papilloma. Cluster of cells with cytoplasmic pallor, HE, 200x.

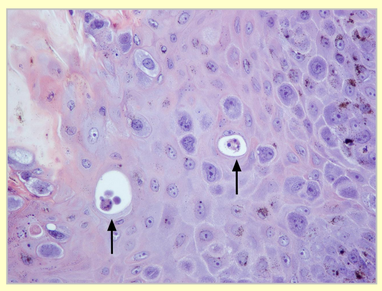

Fig. 6: Plaque. Note two koilocytes (arrow) characterised by a clear cytoplasm and pyknotic nuclei, HE,400x.

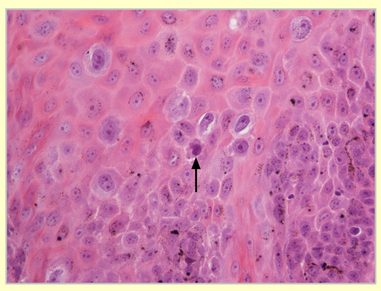

Fig. 7: papilloma. Scattered basophilic intranuclear inclusion body (arrow), HE, 400x.

PCR

In situ hybridisation

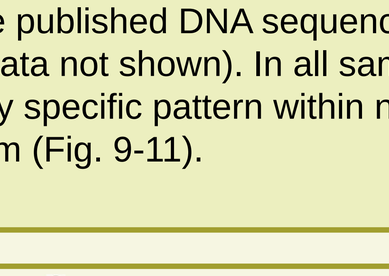

Fig. 8 Agarose gel electrophoresis. PCR of amplified COPV-DNA for 261 bp L1 (1-3), 350 bp E6 (4-6), 204 bp E7 (7-9); lane 1,4,7 plaque, dog No.1; lane 2,5,8 papilloma, dog No.2; lane 3,6,9 papilloma, dog No.3.

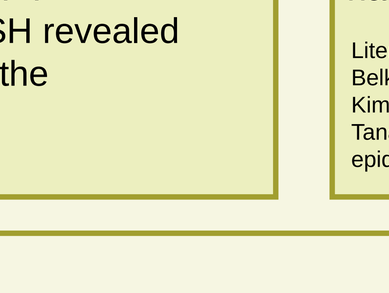

Fig. 9 Papilloma, dog No. 2. Nucleus associated hybridisation signals specific for COPV- DNA in epithelial cells of stratum spinosum and corneum, 20x.

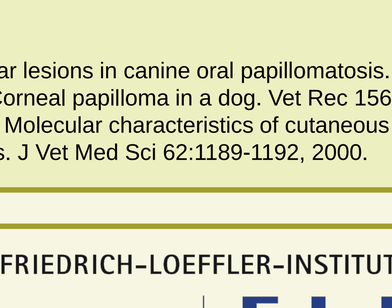

Fig. 10: Plaque. Intranuclear ISH signal for COPV-DNA within upper epithelial layers, 200x.

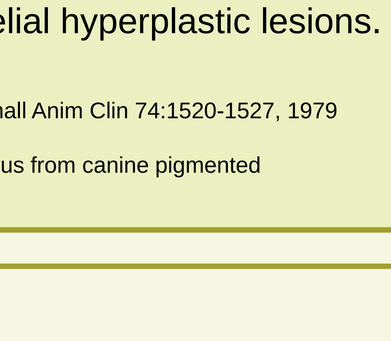

Fig. 11 plaque. Nucleus associated hybridisation signal for COPV- DNA, in keratinocytes and koilocytes, 400x.

Results:
All three conjunctival neoplasms revealed various degrees of epithelial hyperplasia, acanthosis and hyperkeratosis with hypergranulosis and koilocytosis in the upper epithelial layers. Occasionally, basophilic intranuclear inclusions were present (Fig. 4-7). Based on the different morphological aspects, including the growth pattern and surface aspect conjunctival plaque and viral papillomas were diagnosed. In all three lesions PCR for COPV-DNA yielded amplification products each with the predicted size for the E6, E7 and L1 genes (Fig. 8). Sequencing of the amplicons revealed for all three fragments 100% identity with the published DNA sequence of COPV (Genbank: D55633, data not shown). In all samples, ISH revealed COPV-DNA in a highly specific pattern within nuclei of the hyperplastic epithelium (Fig. 9-11).

Discussion:
Based only on the different morphology, consideration must be given to whether the plaque is a preliminary stage of an exophytic papilloma or an entity on its own. The molecular results, however, support the assumption that the plaque is the initial lesion progressing into papilloma with time. Since COPV was isolated from squamous cell carcinomas, a progression from virus-induced papilloma or plaque to this malignant variant must be also taken into consideration. In contrast to this conjunctival plaque a papilloma virus different from COPV was isolated from viral skin plaques. This is the first time that the oncogenic lambdapapillomavirus COPV has been detected in ocular mucosal epithelial hyperplastic lesions.

Literature:
Belkin VP: Ocular lesions in canine oral papillomatosis. Vet Med Small Anim Clin 74:1520-1527, 1979
Kim MS et al.: Corneal papilloma in a dog. Vet Rec 156:454, 2005.
Tanabe C et al.: Molecular characteristics of cutaneous papillomavirus from canine pigmented epidermal nevus. J Vet Med Sci 62:1189-1192, 2000.

Tierpathologie Augsburg

FRIEDRICH-LOEFFLER-INSTITUT
FLI
Bundesforschungsinstitut für Tiergesundheit