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GENOME ANNOUNCEMENT

Entire Genomic Sequence of Novel Canine Papillomavirus Type 13

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Papillomaviruses are associated with benign and malignant neoplasias of the skin and mucous membranes. The sequence of a novel canine papillomavirus was determined from DNA detected in the oral cavity of a dog. The sequence of the novel virus canine papillomavirus type 13 (CPV13) shares the highest levels of similarity with the Tau papillomaviruses CPV2 and CPV7.

Papillomaviruses are nonenveloped, icosahedral particles, approximately 50 nm in diameter, with a circular, double-stranded DNA genome of about 8,000 bp. Typically, they are host species-specific and tissue-restricted putative pathogens. Many of the known papillomaviruses are associated with benign and malignant neoplasias of the keratinizing and nonkeratinizing skin in humans and animals (3). More than 150 human papillomavirus types and also many animal papillomavirus types have been discovered, illustrating a broad genetic diversity (1, 3, 4). A dozen complete and a few partial canine papillomavirus (CPV) sequences have been published and linked to various neoplasias. Thus far, all CPVs were allocated to three different papillomavirus genera, i.e., Lambda, Tau, or Chi (1, 2, 5–14).

A cytobrush sample of a mixed-breed dog showing symptoms of oral papillomatosis was taken for diagnostic purposes. Total DNA was isolated from the sample, and a circular DNA was amplified by rolling circle amplification (RCA) and partially as well as entirely cloned into the BamHI, ClaI, or EagI site of a pBluescript II KS+ vector (Stratagene). The sequences of the RCA product and the genomic clones were determined independently using an ABI 377 (Applied Biosystems) sequencer, and primary sequences were assembled using ContigExpress software (Vector NTI Informax; Invitrogen), revealing a papillomavirus genome of 8,228 bp with a GC content of 47%. Pairwise sequence alignments were performed with the obtained sequence using the Needleman-Wunsch algorithm, and a phylogenetic tree was calculated based on the coding sequences for the E6, E7, E1, E2, L2, and L1 proteins (data not shown) (7).

The characteristic open reading frames E1, E2, E4, E6, E7, L1, and L2 and two noncoding regions between L1 and E6 (478 bp) and between E2 and L2 (914 bp) were identified on the nucleotide sequence of the novel isolate. Dyad symmetry repeats (TTGTTG TTAACAACAA) in a modified form flanked by E2 binding sites (ACC-N_6-GGT) were located about one hundred nucleotides upstream of E6, putatively marking the origin of replication. Polyadenylation signals (AATAAA) were found near the 5′ end of the L2 open reading frame as well as in the terminal third and at the 3′ end of the L1 open reading frame. A typical pRB binding motif (LXCXE) in the E7 amino acid sequence of the novel isolate was not detected, an absence shared with the Tau papillomaviruses CPV2 and CPV7.

The L1 nucleotide sequence shared 63% identity with CPV7 and 62% identity with CPV2, while it shared less than 60% identity with all other published papillomavirus L1 sequences. The highest degrees of similarity for the E1 amino acid sequence were found with CPV7 (68.8%) and CPV2 (68.4%). On the level of E6 and E7, the highest similarities were observed with CPV2 (55.3% and 68.4%, respectively) and CPV7 (50.4% and 64.3%, respectively). Upon the phylogenetic analysis, the novel papillomavirus sequence clustered with the two previously described Tau CPVs. Based on the overall analyses, the new isolate was designated CPV13.

Taken together, the findings suggest that CPV13 might be the third member of the papillomavirus genus Tau, putatively establishing a new species within it.

Nucleotide sequence accession number. The nucleotide sequence data of CPV13 were deposited in GenBank under accession number JX141478.

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