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Short communication

Comparison of genetic characteristics of canine papillomaviruses in Turkey

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ABSTRACT

Papillomavirus (PV) infections often cause benign and malignant skin neoplasia in dogs. To date, twenty types of canine papillomaviruses (CPVs) have been described worldwide. A detailed molecular characterization of CPVs in Turkey is lacking. In the present study, oral and mucosal lesions from 13 dogs with suspected CPV infection from the Mediterranean and central Anatolian regions of Turkey were analyzed. The partial gene sequences of the L1, E6, and E7 regions were compared with those of CPV types in the GenBank database. The results showed that CPV-1 infection was the dominant type of canine papillomatosis in Turkey. In addition, there was no statistically significant association between the frequency of the disease and the age or gender of the dog ($p > 0.05$). However, all the dogs were pedigree breeds, suggesting that the disease may be more prevalent among pure-bred dogs than mixed breeds.

1. Introduction

A number of canine papillomaviruses (CPVs) that infect dogs have been identified in recent years (Rector and Van Ranst, 2013). These viruses cause contemporaneous immunosuppression and cutaneous/or oral warts/papillomas in infected animals. To date, 49 different genera in International Committee on the Taxonomy of Viruses (ICTV) have been described belonging to the family *Papillomaviridae*, and twenty CPV types have been distributed based on their genomic criteria into three genera *Lambdapapillomavirus* (types 1 and 6), *Taupapillomavirus* (types 2, 7, 13, 17 and 19), and *Chipapillomavirus* (types 3, 4, 5, 8, 9, 10, 11, 12, 14, 15, 16, 18 and 20) (Rector and Van Ranst, 2013; Christensen et al., 2016; Gil da Costa et al., 2016; Lange et al., 2009; Munday et al., 2016; Tisza et al., 2016).

There are eight genes (E6, E7, E1, E2, E4, E5, L2, and L1) in the double-stranded DNA genome of canine papillomavirus (PV) (de Villiers et al., 2004). L1 encodes a structural protein, namely capsid protein. Current vaccines are based on virus-like particles produced by the L1 gene (Suzich et al., 1995; Stanley et al., 2001). Additionally, the L1 gene is useful for the classification of PVs and necessary to obtain phylogenetic information about PVs (Bernard et al., 2010). E6 and E7 are “adaptive proteins” and have various functions, including modulating cellular growth and immune responses (Klingelhutz and Roman, 2012). The E6 and E7 genes play important roles in viral evolution studies, PV oncogenesis, and typing of PVs (Ghittoni et al., 2010; van Doorslaer, 2013; van Doorslaer et al., 2013).

Thus far, studies of CPV in Turkey have focused only on the detection and treatment of this infection (Biricik et al., 2008; Yagci et al., 2008; Sancak et al., 2015). The first aim of the current study was to determine the molecular types of PV based on L1, E6, and E7 partial gene sequences circulating among domestic dogs in Turkey. The second aim was to determine the statistical significance of any relation between emergence of infection and animal features (age, gender, and breed) by comparing CPV-positive domestic dogs.

2. Materials and methods

2.1. Animals and sample collection

Skin samples for biopsies were collected from 13 dogs with a clinical suspicion of PV infection. The samples were collected at veterinary clinics and sent to our laboratory for diagnostic analysis. The samples were stored at − 20 °C in PBS. Information on the age, gender, and breed of each animal is presented in Table 1. Chi-square analysis was performed for statistically analysis.

2.2. DNA extraction and PCR

DNA from tissue homogenates were extracted using a commercial kit (High Pure Viral Nucleic Acid Extraction Kit, Roche, Product No: 11,858,874,001, Mannheim, Germany) according to the instructions of the manufacturer. Nucleic acid samples were then kept at − 20 °C until

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Table 1
Information about sampled animals.

ID	Breed	Age	Gender	Location of lesions	E6 accession numbers	E7 accession numbers	L1 accession numbers
TR-CanPV-1	British setter	11 m	F	Cutaneous	KY465872	KY486637	KY445587
TR-CanPV-2	Golden retriever	8 m	F	Oral	KY465873	KY486638	KY445588
TR-CanPV-3	Boxer	16 m	M	Cutaneous	KY465874	KY486639	KY445589
TR-CanPV-4	Pointer	5 m	F	Oral	KY465875	KY486640	KY445590
TR-CanPV-5	Golden retriever	8 m	F	Oral	KY465876	KY486641	KY445591
TR-CanPV-6	Terrier	1.5 a	F	Oral	KY465877	KY486642	KY445592
TR-CanPV-7	Husky	1.5 a	M	Cutaneous	KY465878	KY486643	KY445593
TR-CanPV-8	Terrier	1 a	F	Cutaneous	KY465879	KY486644	KY445594
TR-CanPV-9	Bulldog	2 a	M	Cutaneous	KY465880	KY486645	KY445595
TR-CanPV-10	Golden retriever	1 a	M	Oral	KY465881	KY486646	KY445596
TR-CanPV-11	Cane Corso Italiano	8 m	M	Cutaneous	KY465882	KY486647	KY445597
TR-CanPV-12	Golden retriever	7 m	M	Oral	KY465883	KY486648	KY445598
TR-CanPV-13	Danua	1 a	F	Oral	KY465884	KY486649	KY445599

m: month, a: age, F: female, M: male.

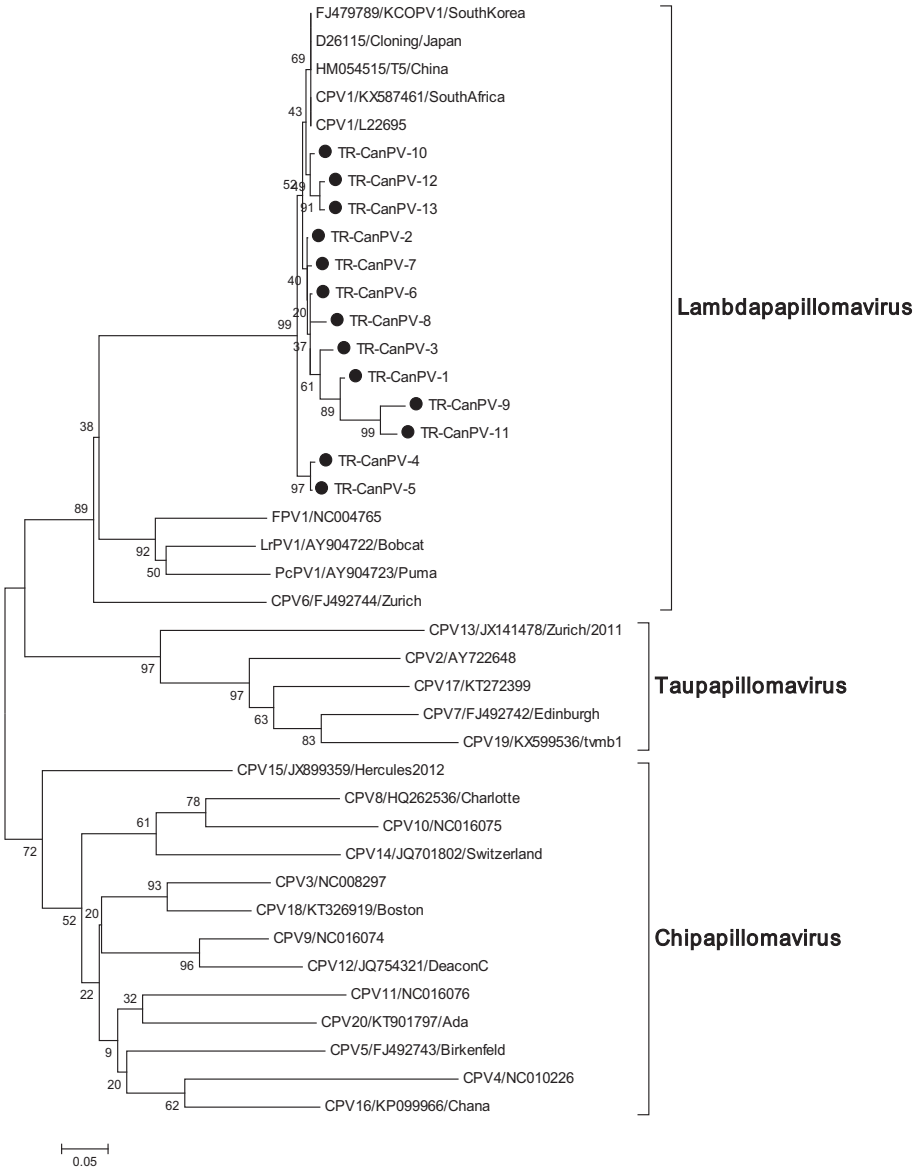

Fig. 1. Phylogenetic trees based on the all canine papillomaviruses, nucleotide sequences of partial L1 gene.

the PCR application. For amplification of Papillomavirus DNAs E6 (350 bp), E7 (208 bp) and L1 (261 bp), genes primers were used described by Teifke et al. (1998). PCR were performed according to the protocol in same publication with minor modifications especially in annealing temperatures, for E6 at 50 °C, for E7 at 52 °C and for L1 at 55 °C.