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NEOPLASTIC DISEASE

Absence of Canine Papillomavirus Sequences in Canine Mammary Tumours

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Summary

Human papillomaviruses (PVs) are found in human breast cancer tissue; however, it remains controversial as to whether these viruses play a role in the aetiology of this tumour. There has been minimal study of whether PVs are found in normal or abnormal mammary glands of animals. The present study investigated whether a PV sequence could be found in the mammary glands of 33 female dogs by rolling circle amplification and polymerase chain reaction. No PV DNA was found in normal or neoplastic canine mammary tissues, suggesting that canine PVs are probably not involved in the pathogenesis of canine mammary neoplasia.

Keywords: dog; mammary tumour; papillomavirus

Papillomaviruses (PVs) are small, non-enveloped, double-stranded DNA viruses with a circular genome of about 8,000 base pairs (bps). They are a genetically diverse group of viruses and their number is constantly increasing, with more than 200 human papillomavirus (HPV) types and many animal PVs identified to date (Bernard *et al.*, 2010; Rector and Van Ranst, 2013; Lecis *et al.*, 2014). PVs are predominantly species-specific and tissue-restricted pathogens that, due to a tropism for the basal layer of skin and mucous membrane epithelia, are associated with distinct pathological entities including single or multiple epithelial plaques, papillomas and carcinomas in keratinizing and non-keratinizing epithelia (Lange *et al.*, 2011). In human medicine, HPV 16 is the most prevalent type involved in the pathogenesis of human cervical carcinomas (Doorbar, 2006), while many other HPVs induce benign papillomas of skin and mucous membranes.

Canine PV (CPV) infection may lead to the formation of papillomas affecting the skin and mucous membranes (e.g. oral, penile, vulval and conjunctival) (Nicholls and Stanley, 1999). According to the Papillomavirus Episteme (PAVE) the complete genomic sequence of 15 CPVs has been obtained to date. Additionally, several partial CPV sequences have been published and linked to various tumours (Lange *et al.*, 2012).

Mammary gland tumours are one of the most common neoplasms in female dogs and their epidemiological, morphological and molecular characteristics, as well as their biological behaviour, are similar to those of breast carcinomas in women (Antuofermo *et al.*, 2007; Sleeckx *et al.*, 2011; Pinho *et al.*, 2012). The incidence of mammary tumours in bitches is three times greater than in women (Egenvall *et al.*, 2005) and approximately half of affected dogs have malignant tumours (Sorenmo, 2003). The aetiopathogenesis of canine mammary neoplasia likely involves the complex interactions of multiple factors including environmental, nutritional and infectious factors. In the case of the latter factor, oncogenic viruses have been suggested to play an important role (Alenza *et al.*, 2000).

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Table 1
Histological and molecular characterization of canine mammary samples

Dog number	*Breed*	*Age (years)*	*Histological diagnosis*	*RCA*	*PCR*
1	Dachshund	4	Normal	Negative	Negative
2	Mixed breed	13	Normal	Negative	Negative
3	Yorkshire terrier	8	Normal	Negative	Negative
4	Mixed breed	11	Normal	Negative	Negative
5	Labrador retriever	9	Normal	Negative	Negative
6	Yorkshire terrier	8	Complex adenoma	Negative	Negative
7	German shepherd dog	10	Complex adenoma	Negative	Negative
8	Mixed breed	10	Complex adenoma	Negative	Negative
9	Mixed breed	6	Benign mixed tumour	Negative	Negative
10	Yorkshire terrier	8	Complex adenoma	Negative	Negative
11	Mixed breed	11	Complex carcinoma	Negative	Negative
12	Mixed breed	14	Complex carcinoma	Negative	Negative
12*			Complex carcinoma	Negative	Negative
13	German shepherd dog	14	Complex carcinoma	Negative	Negative
14	Yorkshire terrier	15	Complex carcinoma	Negative	Negative
14*			Simple carcinoma	Negative	Negative
15	Yorkshire terrier	9	Simple carcinoma	Negative	Negative
16	Mixed breed	13	Simple carcinoma	Negative	Negative
17	German shepherd dog	13	Simple carcinoma	Negative	Negative
18	Mixed breed	11	Simple carcinoma	Negative	Negative
19	Yorkshire terrier	13	Complex carcinoma	NT	Negative
20	German shepherd dog	12	Simple carcinoma	NT	Negative
21	Golden retriever	11	Benign mixed tumour	NT	Negative
22	Shih Tzu	6	Complex adenoma	NT	Negative
23	Mixed breed	8	Complex carcinoma	NT	Negative
24	Labrador retriever	9	Complex carcinoma	NT	Negative
24*			Complex carcinoma	NT	Negative
25	Bobtail	9	Simple carcinoma	NT	Negative
26	Golden retriever	13	Complex carcinoma	NT	Negative
27	Bobtail	6	Complex carcinoma	NT	Negative
27*			Complex carcinoma	NT	Negative
28	German shepherd dog	12	Complex carcinoma	NT	Negative
28*			Complex carcinoma	NT	Negative
29	Mixed breed	11	Complex carcinoma	NT	Negative
30	Cocker spaniel	11	Benign mixed tumour	NT	Negative
31	Mixed breed	13	Simple carcinoma	NT	Negative
32	Poodle	9	Complex carcinoma	NT	Negative

*Dog with multiple lesions; RCA, rolling circle amplification; PCR, polymerase chain reaction; NT, not tested.

Recently, HPVs have been identified in 46% of cases of human breast ductal carcinoma in situ and in 28% of invasive breast carcinomas (Glenn *et al.*, 2012). In line with these studies, Li *et al.* (2011) conducted a meta-analysis in which it was shown that 24.5% of breast carcinomas were associated with HPV, of which 32.4% occurred in Asia and 12.9% in Europe. The most prevalent HPV types identified in breast cancer from different populations are HPVs 33, 18, 16 and 35 and women with HPV-positive breast cancer are significantly younger than those negative for HPV (Li *et al.*, 2011).

Conversely, there are no reported studies concerning the involvement of PVs in canine mammary gland lesions. The aim of this study was to determine whether CPVs could be found in mammary tissue obtained from dogs in two geographically distant regions in order to investigate a possible association between CPV and mammary cancer.

Thirty-seven mammary gland samples, collected from 32 female dogs, were obtained from Spain (Veterinary Hospital of Alfonso X El Sabio University) and Italy (Veterinary Teaching Hospital, Sassari University, Sardinia) and submitted for histopathological and molecular examinations (Table 1). Specimens were halved and one half was stored at −80°C for molecular analysis while the other was fixed in 10% neutral buffered formalin. Fixed tissue was processed routinely, embedded in paraffin wax and sections (3 μm) were stained with haematoxylin and eosin (HE). Mammary tissues were classified according to the criteria of the World Health Organization (Misdorp *et al.*, 1999). The samples included tissue