



Nasal virome of dogs with respiratory infection signs include novel taupapillomaviruses

Eda Altan^{1,2} · M. Alexis Seguin³ · Christian M. Leutenegger⁴ · Tung Gia Phan^{1,2} · Xutao Deng^{1,2} · Eric Delwart^{1,2}

Received: 2 November 2018 / Accepted: 4 January 2019 / Published online: 10 January 2019
© Springer Science+Business Media, LLC, part of Springer Nature 2019

Abstract

Using viral metagenomics, we characterized the mammalian virome of nasal swabs from 57 dogs with unexplained signs of respiratory infection showing mostly negative results using the IDEXX Canine Respiratory Disease RealPCR™ Panel. We identified canine parainfluenza virus 5, canine respiratory coronavirus, carnivore bocaparvovirus 3, canine circovirus and canine papillomavirus 9. Novel canine taupapillomaviruses (CPV21–23) were also identified in 3 dogs and their complete genome sequenced showing L1 nucleotide identity ranging from 68.4 to 70.3% to their closest taupapillomavirus relative. *Taupapillomavirus* were the only mammalian viral nucleic acids detected in two affected dogs, while a third dog was coinfecting with low levels of canine parainfluenza 5. A role for these taupapillomaviruses in canine respiratory disease remains to be determined.

Keywords Papillomaviridae · Papillomavirus · Taupapillomavirus · Canine · Next generation sequencing · Respiratory infection

Introduction

Canine infectious respiratory disease (CIRD) is a major cause of morbidity and an important welfare issue for kennelled dog populations worldwide. The canine respiratory disease is generally characterized by coughing, snorting, wheezing, sneezing, nasal and eye discharge, fever, and dry and itchy nose. In some cases, the disease can be lethal if bronchopneumonia develops [1]. Viral causes for respiratory disease in dogs include canine adenovirus [2], canine

respiratory coronavirus, canine herpes virus, distemper virus [3], influenza virus [4], canine parainfluenza virus [1] and a recently described canine protoparvovirus [5].

The *Papillomaviridae* family currently consists of at least 53 genera formed by 133 papillomavirus (PV) species that have been identified in humans, non-human mammals, birds, reptiles and fish [6]. Transmission of warts between dogs was first shown in 1898 [7] and cell-free extracts were shown to transmit canine papillomavirus (CPV) in 1959 [8]. Canine oral papillomas are typically benign and rapidly regress rarely causing respiratory problems [8] except in immuno-suppressed dogs which can develop severe oral papillomatosis [9–11].

The first CPV genome was sequenced in 1986 [12]. To date a total of 20 CPV have been described that can be classified into 3 different genera [13–16]. Most recently canine papillomavirus type 18 genome was characterized in pigmented plaques [17], canine papillomavirus 19 was sequenced from an oral papilloma coinfecting with CPV1 and CPV2 [16], and the genome of canine papillomavirus 20 was released in 2016 (GenBank accession: KT901797). Of these 20 canine papillomavirus genomes, five are classified in the *Taupapillomavirus* genus (CPV 2,7,13,17,19), 2 in the lambda genera (CPV 1,6) and 13 in the Chi genera (CPV 3,4,5,8,9,10,11,12,14,15,16,18,20). *Lambdapapillomavirus*

Edited by Simon D. Scott.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s11262-019-01634-6>) contains supplementary material, which is available to authorized users.

✉ Eric Delwart
Eric.Delwart@ucsf.edu

¹ Vitalant Research Institute, 270 Masonic Ave, San Francisco, CA, USA

² Department of Laboratory Medicine, University of California San Francisco, San Francisco, CA, USA

³ IDEXX Laboratories, Inc., Westbrook, ME 04092, USA

⁴ IDEXX Laboratories, Inc., West Sacramento, CA 95605, USA

CPV1 has been shown to cause oral papillomatosis [18]. CPV2, 7, 13, 17 and 19 within the *taupapillomavirus* genus were reported in endophytic and exophytic papillomas, squamous cell carcinoma, oral papillomatosis and oral squamous cell [16, 17, 19, 20].

Here we characterized the eukaryotic virome of nasal swabs from dogs with unexplained respiratory infection signs and characterize three novel taupapillomavirus genomes.

Materials and methods

Canine samples

Nasal swab samples were collected from dogs with unexplained respiratory disease. With a few exceptions discussed below, these samples had pre-tested negative for IDEXX Canine Respiratory Disease RealPCR™ Panel. A total of 57 nasal swab samples from different dogs were first analyzed in 11 pools of 1–7 samples using a viral metagenomic approach.

Viral metagenomics

Nasal swab samples were diluted with 750 µl Dulbecco's phosphate-buffered saline, pooled, clarified by 15,000 g centrifugation for 5 min, and supernatants filtered using a 0.45-µm filter (Millipore). Free nucleic acids in the filtrates (not protected in viral capsids) were digested using DNase and RNase enzymes to enrich for viral nucleic acids. Nucleic acids were then extracted (MagMAX Viral RNA Isolation Kit, Ambion, Inc, Austin, Tx, USA) [21] and amplified by random RT-PCR followed by use of the Nextera™ XT Sample Preparation Kit (Illumina) to generate a library for Illumina MiSeq (2 × 250 bases) with dual barcoding as previously described [22].

Following de novo assembly using the Ensemble program [23], both contigs and singlets viral sequences were then analyzed using translated protein sequence similarity search (BLASTx v.2.2.7) to all annotated viral proteins available in GenBank. Geneious R10 was then used to align reads and contigs to reference viral genomes from GenBank the program.

Generation of full genomes of taupapillomaviruses

Back to back (inverse) PCR primers were designed based on metagenomics-derived sequences matching papillomaviruses (Supplementary material 1). PCR was carried out in a total volume of 25 µl. PCR mixes contained 3 µl of extracted DNA, 1.25 units Takara La Taq DNA polymerase (Takara), 10 pmol of each primer sets dNTP mixture (Takara) and the

supplied 10X La PCR buffer II (Takara). First round PCR products were used as template in a 25 µl nested PCR. After an initial denaturation at 94 °C for 1 min, amplification was performed for 40 cycles consisting of 20 s at 94 °C, 30 s at 55 °C and 2 to 7 min (depending on PCR product size) at 72 °C, followed by a final extension for 10 min at 72 °C. Long PCR amplicons were sequenced using the Nextera library making kit and the MiSeq Illumina sequencer. PCR primers used to identify individual taupapillomavirus positive samples in the pool samples are described (Supplementary material 1).

Phylogenetic analysis

The E1-E2-L1-L2 protein sequences were aligned using MAFFT [24, 25] in Geneious v10.1.3., and phylogenetic tree was derived using the maximum likelihood method based on the general time reversible model [26]. The percentage of trees in which the associated taxa clustered together is shown next to the branch points. Initial tree(s) for the heuristic search were obtained by applying the neighbor-joining method to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 27 nucleotide sequences of all currently known canine papillomaviruses. Positions containing gaps and missing data were eliminated. There were a total of 5051 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 [27].

The L1 gene nucleotide sequences were aligned using MAFFT [24, 25] in Geneious v10.1.3., and phylogenetic tree was derived using the neighbor-joining method [28]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the maximum composite likelihood method [29] and are in the units of the number of base substitutions per site. The analysis involved 27 nucleotide sequences. There were a total of 1423 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 [27].

Results

Viral metagenomic

Mammalian viruses detected with BLASTx-translated protein matches (E score < 10⁻³⁰) were: rubulavirus 5 (also known as canine parainfluenza virus 5 or PIV5) in 3 different pools, carnivore bocaparvovirus 3 in two pools, canine betacoronavirus in one pool, canine circovirus in one pool, and canine papillomavirus 9 (Chi genus) in one pool (Table 1). Three pools also contained reads showing E score < 10⁻³⁰

Table 1 Distribution of sequence reads to different viruses in 11 nasal swab pools and 3 individual taupapillomavirus positive swab samples

| | No. of sample in pool | Total reads | Circoviridae | Paramyxoviridae | Papillomaviridae | | Parvoviridae | Coronaviridae |
|---------------------------------------|-----------------------|-------------|--------------------|-----------------------|--------------------|---------------------|-----------------------------|--------------------|
| | | | Canine circo-virus | Parainfluenza virus 5 | Taupapilloma-virus | Chi papil-lomavirus | Carnivore bocaparvovi-rus 3 | Betacoronavi-rus 1 |
| Pool1 | 5 | 497,208 | | 20 | 29,029 | | | |
| Pool2 | 5 | 1,516,776 | | | | 131 | 2 | |
| Pool3 | 5 | 523,486 | | | | | 2 | |
| Pool4 | 5 | 2,493,284 | | | | | | |
| Pool5 | 5 | 667,534 | | | | | | |
| Pool6 | 6 | 606,050 | | 8 | | | | |
| Pool7 | 7 | 1,125,636 | | | | | | |
| Pool8 | 7 | 673,820 | | | | | | |
| Pool9 | 7 | 674,772 | | | 287 | | | |
| Pool10 | 1 | 1,107,958 | | | 665 | | | |
| Pool11 | 4 | 930,598 | 4278 | 31 | | | | 11 |
| Case 1 (indi-vidual sample in Pool1) | | 202,354 | | 8 | 3084 | | | |
| Case 2 (indi-vidual sample in Pool9) | | 353,980 | | | 2424 | | | |
| Case 3 (indi-vidual sample in Pool10) | | 399,948 | | | 1812 | | | |

to taupapillomaviruses (Table 1). PCR based on these taupapillomavirus sequences were first used to identify the individual positive samples within each of the three pools (Supplementary material 1). These three samples were then deep sequenced individually (rather than in pools) to confirm the presence of the taupapillomavirus, acquire more of their genome sequences and test for coinfecting viral genomes.

Taupapillomavirus positive animal

Clinical case 1 (in pool 1) shedding taupapillomavirus CPV21 was a 12-month-old intact male Labrador retriever with respiratory infection signs. The nasal swab of case 1 was tested by the IDEXX Canine Respiratory Disease Real-PCR™ Panel consisting of real-time PCRs for *Bordetella bronchiseptica*, canine adenovirus type 2, canine distemper virus, canine herpesvirus type 1, canine parainfluenza virus, canine pneumovirus, canine respiratory coronavirus, H3N2 canine influenza virus, other influenza A virus (H1N1, H3N8, H7N2), *Mycoplasma cynos*, and *Streptococcus equi subsp. zooepidemicus*. Case 1 was positive only for *Mycoplasma cynos*. Two other dogs from the same household also exhibited similar respiratory infection signs. One of these dogs was also tested with the IDEXX Canine Respiratory Disease RealPCR™ Panel with negative results for all agents. Swabs from both of these related dogs tested

negative for taupapillomavirus CPV21 by both metagenomic sequencing and PCR.

Clinical case 2 (in pool 9) infected with CPV22 was an eleven months old, intact female Miniature Australian Shepherd with sneezing and coughing since boarding at a clinic. All tests on the IDEXX Canine Respiratory Disease Real-PCR™ Panel were negative.

Clinical case 3 (in pool 10) infected with CPV23 was a 1-year 2-month-old male Pembroke Welsh Corgi. This animal was part of an outbreak of tracheobronchitis in up to 20 dogs in a canine daycare center with upper respiratory infection signs of coughing with occasional clear phlegm/saliva. No ocular or nasal discharges were reported. All tests on the IDEXX Canine Respiratory Disease RealPCR™ Panel on case 3 were negative. The same respiratory panel was also used on two other patients in same outbreak which were positive for *Mycoplasma cynos* only. Neither of these two nor other animals from that outbreak were available for metagenomics or taupapillomavirus PCR.

Results of full genomes generation

Two to four hundred thousand sequence reads were generated for each of the three individual respiratory samples (Table 1). The raw sequence data for each of these 3 nasal

swab samples are available at NCBI's Short Reads Archive under GenBank accession number SRP133969.

The most commonly detected eukaryotic viral reads in these three nasal swabs belonged to the *Taupapillomavirus* genus in the *Papillomaviridae* family. The frequency of reads belonging to the assembled genomes ranged from 0.45 to 1.5% of the total read numbers. One swab from case 1 also contained nucleic acids from another canine virus, canine parainfluenza virus 5 (PIV5) at the low read frequency of 0.0032% (8 out of 202,354 reads).

Taupapillomavirus genomes

Gaps in the assembled genome sequences were filled by long-range PCR using the primers described (Supplementary material 1). The full genome of the canine taupapillomaviruses was 8140 (CPV23), 8300 (CPV22) and 8225 bp (CPV21) in length, with 46.8 to 48.40% GC content coding for five early proteins (E1, E2, E4, E6 and E7) and two late proteins (L1 and L2) (GenBank accession numbers; MH285952 for CPV21, MH285953 for CPV22, MH285954 for CPV23, Supplementary material 2). The predicted ORFs and characteristics of their putative protein products are shown in supplementary material 3. As customary the 'A' in the first start codon of the ORF E6 was assigned position 1. The ORF genetic organization of CPV21–23 was identical to that of other taupapillomaviruses.

All E6 and E7 proteins showed conserved zinc-binding domains (two domain CXXC-X29-CXXC for E6 and single domain CXXC-X30-CXXC for E7) but lacked for PDZ-binding motif (ETQL) and retinoblastoma (pRb) protein-binding site that is present in high-risk oncogenic PVs [30] although it has been shown for canine papillomavirus 2 that a distinct region is responsible for pRb binding and degradation [31]. The long control region [32] between E2 and L2 was 648, 714 and 842 bases long. Conserved helicase domain, the conserved ATP-binding site of the ATP-dependent helicase (GXXXXGK[T/S]), cyclin A interaction motifs and the leucine–zipper domain were identified within the hypothetical proteins (Supplementary material 4).

Maximum likelihood (ML) tree based on the concatenated alignment of E1, E2, L1 and L2 from new taupapillomavirus tree with bootstrap support for the position of the branches is shown in Fig. 1a. The novel taupapillomaviruses clustered with the *Taupapillomavirus* genus with high bootstrap support.

The three L1 ORF showed nucleotide identity of 67.3 to 69.6% to each other and from 68.4 to 70.3% with their closest relative, canine papillomavirus 13 isolate Zurich/2011 (CPV13, GenBank accession number JX141478) [20] and 52 to 63.5% similarity with other canine papillomavirus L1 (Table 2).

According to the phylogenetic tree constructed with the L1 nucleotide region, the papillomavirus genomes in nasal swab clustered with the taupapillomaviruses, most closely with CPV13 (described in 2011 from a dog with papillomatosis) [16, 17, 19, 20] (Fig. 1b). The suggested ICTV criteria for naming a new genera require an L1 nucleotide identity of approximately < 60% [33–35]. A distribution of pair-wise distances shows that L1 sequences classified with the same species generally showed identity of 70–100% [33, 34]. Given these criteria, the latest ICTV release for the *Papillomaviridae* family (June 2018) list four species in the *Taupapillomavirus* genus. *Taupapillomaviruses* 1 and 2 consist of only canine viruses. *Taupapillomavirus* 3 is represented by feline viruses (FcaPV3, FcaPV4) [36, 37] and *Taupapillomavirus* 4 by a ferret papillomavirus (MpPV1) [38, 39] (Fig. 1). The L1 nucleotide identity between the new canine taupapillomaviruses described here and their closest relative, CPV13 in *taupapillomavirus* species 2, ranged from 68.4 to 70.3% (Table 2). The new taupapillomaviruses described here (CPV21–23) can therefore most conservatively be considered new types within *Taupapillomavirus* 2 species.

Discussion

We analyzed the virome of nasal swabs from fifty-seven dogs with unexplained respiratory infection signs. Reads from three novel taupapillomaviruses were identified and their complete genomes sequenced (CPV21–23). Genomes of canine parainfluenza 5, carnivore bocaparvovirus 3, canine betacoronavirus, canine circovirus, and canine papillomavirus 9 were also detected. All of these viruses except canine circovirus and canine papillomavirus 9 have already been associated with canine respiratory disease although with different level of pathogenicity [40].

It is not known whether papillomatosis was present in the 3 taupapillomavirus containing clinical cases described here. Papillomavirus-induced signs such as epithelial hyperplasia may not be readily visible unless worsened to the level of papillomatosis due to immune deficiency [41, 42]. For clinical case 1, the taupapillomavirus could only be detected by PCR in one of three dogs from the same household showing respiratory infection signs indicating that the taupapillomavirus may be unrelated to this small respiratory disease outbreak or that the samples from the other two dogs were collected after CPV21 was cleared. Clinical case 1 also yielded canine parainfluenza 5 at low level (based on low % of reads and lack of detection using the commercial IDEXX PCR assay). This paramyxovirus is a well-documented canine respiratory pathogen [43].

Case 2 was a sporadic case of respiratory infection signs where the only mammalian virus detected by

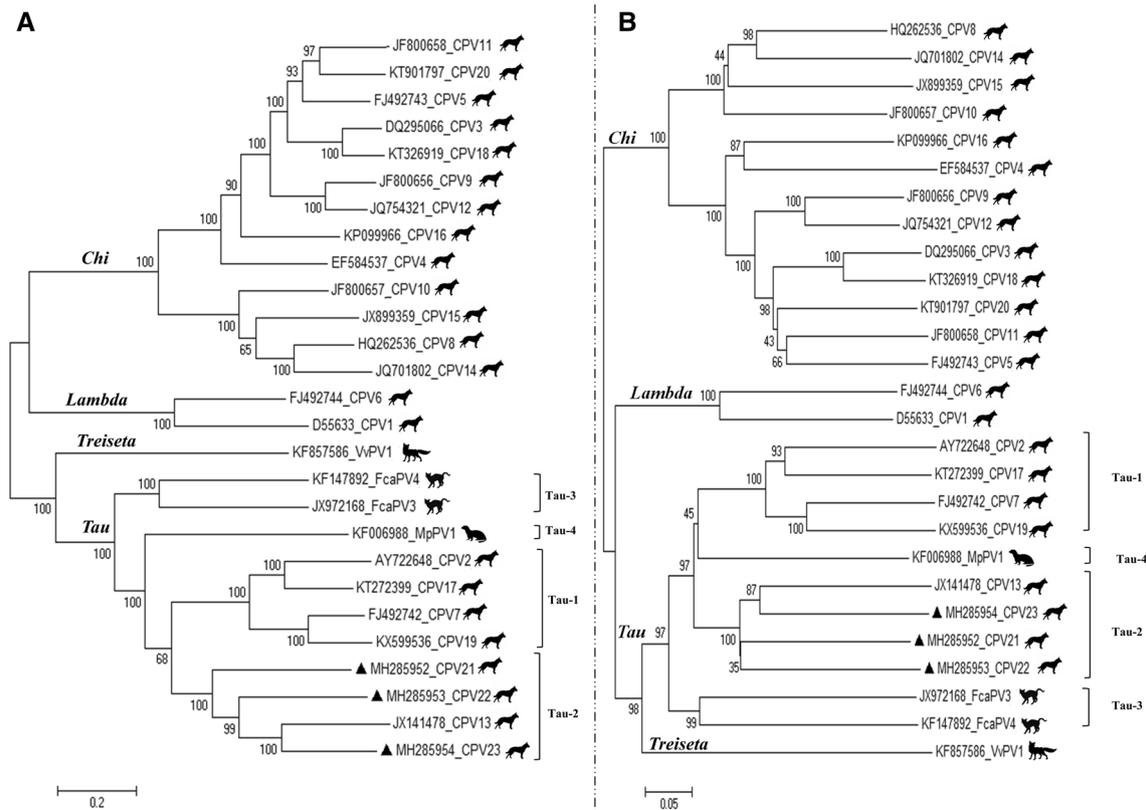


Fig. 1 Phylogenetic analysis of all currently sequenced canine papillomaviruses. Genomes from this study are marked with solid triangles. **a** Maximum likelihood (ML) tree based on the concatenated alignment of E1, E2, L1 and L2 from new taupapilloma virus and 24 other PV types of different species and genera. **b** The L1 gene nucleotide sequences were aligned using MAFFT in Geneious v10.1.3., and phylogenetic tree was derived using the neighbor-joining method. Abbreviations used include canine papillomavirus, CPV; *Felis catus* (cat) papillomavirus, FcaPV; *Mustela putorius* papillomavirus (ferret), MpPV; *Vulpes vulpes* papillomavirus (fox), VvPV

metagenomics was taupapillomavirus. All the commercial IDEXX PCR assays were negative.

Clinical case 3 was part of a larger respiratory outbreak of 20 dogs, three of which were tested on the IDEXX respiratory panel and were negative except for one case of *Mycoplasma cynos*, an infection also commonly seen in asymptomatic dogs [43]. Taupapillomavirus CPV23-infected clinical case 3 was the only dog from that outbreak available for this study.

Evidence for a pathogenic role of taupapillomavirus infection in these 3 dogs with respiratory infection signs therefore remains limited to their detection in nasal swabs in the absence of known respiratory pathogens in 2 of these 3 dogs. Detection of taupapillomavirus DNA may

also reflect frequent asymptomatic or chronic infections unrelated to these animals' respiratory disease. Non-viral pathogens such as untested-for bacteria or fungi may have also been involved in these dog's respiratory signs. Coinfections with parainfluenza 5 and/or *Mycoplasma cynos* may have also worsened the outcome of taupapillomavirus infection as could have immunodeficiency as shown by induction of papillomatosis by other taupapillomaviruses [41, 42].

Further studies will be required to determine the role of taupapillomaviruses in canine respiratory diseases. Such studies may include localizing the precise sites of viral replication in the canine respiratory track using in situ RNA hybridization and of their association with papillomatosis or other respiratory disease signs using matched case-control studies.

Table 2 Percent identity of L1 nucleotide sequences between the tau-papillomaviruses described here and all previously reported canine papillomaviruses

| Accession number_name_genus | CPV21 | CPV22 | CPV23 |
|-----------------------------|--------|--------|--------|
| D55633_CPV1_Lambda | 54.516 | 55.838 | 54.664 |
| AY722648_CPV2_Tau | 60.947 | 59.698 | 59.711 |
| DQ295066_CPV3_Chi | 53.746 | 54.022 | 53.399 |
| EF584537_CPV4_Chi | 52.047 | 52.838 | 52.577 |
| FJ492743_CPV5_Chi | 52.789 | 54.622 | 53.906 |
| FJ492744_CPV6_Lambda | 55.858 | 54.386 | 54.646 |
| FJ492742_CPV7_Tau | 63.57 | 62.187 | 62.788 |
| HQ262536_CPV8_Chi | 54.902 | 54.134 | 54.659 |
| JF800656_CPV9_Chi | 54.142 | 54.879 | 54.42 |
| JF800657_CPV10_Chi | 55.708 | 54.944 | 54.486 |
| JF800658_CPV11_Chi | 54.142 | 54.42 | 53.373 |
| JQ754321_CPV12_Chi | 53.49 | 54.551 | 54.158 |
| JX141478_CPV13_Tau | 68.445 | 69.048 | 70.363 |
| JQ701802_CPV14_Chi | 54.729 | 54.028 | 53.635 |
| JX899359_CPV15_Chi | 55.033 | 54.724 | 56.168 |
| KP099966_CPV16_Chi | 54.929 | 54.581 | 52.381 |
| KT272399_CPV17_Tau | 62.467 | 61.286 | 61.166 |
| KT326919_CPV18_Chi | 52.964 | 53.172 | 52.745 |
| KX599536_CPV19_Tau | 62.846 | 62.582 | 61.012 |
| KT901797_CPV20_Chi | 53.947 | 53.569 | 53.111 |
| MH285952_CPV21_Tau | 100 | 69.625 | 69.226 |
| MH285953_CPV22_Tau | 69.625 | 100 | 67.324 |
| MH285954_CPV23_Tau | 69.226 | 67.324 | 100 |

Acknowledgements We acknowledge the support from Vitalant Research Institute and IDEXX laboratories.

Authors' contributions ED, CML and EA designed the study. EA, TGP and MAS performed the experiments. ED, EA and XD analyzed the data. ED and EA wrote the manuscript. ED and CML edited the manuscript and provided funding. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have conflict of interests.

Ethical approval All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

References

- Appel MJ, Binn LN (1987) Canine infectious tracheobronchitis short review: kennel cough. *Virus infections of carnivores*. Elsevier Science Publishing Co, New York
- Ditchfield J, Macpherson LW, Zbitnew A (1962) Association of canine adenovirus (Toronto A 26/61) with an outbreak of laryngotracheitis (Kennel cough): a preliminary report. *Can Vet J* 3:238–247
- Erls K, Dubovi EJ, Brooks HW, Brownlie J (2004) Longitudinal study of viruses associated with canine infectious respiratory disease. *J Clin Microbiol* 42(10):4524–4529. <https://doi.org/10.1128/JCM.42.10.4524-4529.2004>
- Crawford PC, Dubovi EJ, Castleman WL, Stephenson I, Gibbs EP, Chen L, Smith C, Hill RC, Ferro P, Pompey J, Bright RA, Medina MJ, Johnson CM, Olsen CW, Cox NJ, Klimov AI, Katz JM, Donis RO (2005) Transmission of equine influenza virus to dogs. *Science* 310(5747):482–485. <https://doi.org/10.1126/science.1117950>
- Martella V, Lanave G, Mihalov-Kovacs E, Marton S, Varga-Kugler R, Kaszab E, Di Martino B, Camero M, Decaro N, Buonavoglia C, Banyai K (2018) Novel parvovirus related to primate bufaviruses in dogs. *Emerg Infect Dis* 24(6):1061–1068. <https://doi.org/10.3201/eid2406.171965>
- Taxonomy V (2016) <https://talk.ictvonline.org/taxonomy/>. Accessed February 2 2018
- M'Fadyean J, Hobday F (1898) Note on the experimental transmission of warts in the dog. *J Comp Pathol Ther* 11:341–344. [https://doi.org/10.1016/S0368-1742\(98\)80056-8](https://doi.org/10.1016/S0368-1742(98)80056-8)
- Chambers VC, Evans CA (1959) Canine oral papillomatosis. I. Virus assay and observations on the various stages of the experimental infection. *Cancer Res* 19:1188–1195
- Bredal WP, Thoresen SI, Rimstad E, Aleksandersen M, Nafstad PH (1996) Diagnosis and clinical course of canine oral papillomavirus infection. *J Small Anim Pract* 37(3):138–142
- Le Net JL, Orth G, Sundberg JP, Cassonnet P, Poisson L, Masson MT, George C, Longeat L (1997) Multiple pigmented cutaneous papules associated with a novel canine papillomavirus in an immunosuppressed dog. *Vet Pathol* 34(1):8–14. <https://doi.org/10.1177/030098589703400102>
- Sundberg JP, Smith EK, Herron AJ, Jenson AB, Burk RD, Van Ranst M (1994) Involvement of canine oral papillomavirus in generalized oral and cutaneous verrucosis in a Chinese Shar Pei dog. *Vet Pathol* 31(2):183–187. <https://doi.org/10.1177/030098589403100204>
- Sundberg JP, O'Banion MK, Schmidt-Didier E, Reichmann ME (1986) Cloning and characterization of a canine oral papillomavirus. *Am J Vet Res* 47(5):1142–1144
- Munday JS, Thomson NA, Luff JA (2017) Papillomaviruses in dogs and cats. *Vet J* 225:23–31. <https://doi.org/10.1016/j.tvjl.2017.04.018>
- Zhou D, Paul S, Alkhalawi F, Clark M, Schlegel R, Yuan H (2016) Canine papillomavirus 20 isolate Ada, complete genome. GenBank. <https://www.ncbi.nlm.nih.gov/nucleotide/1008264056>. Accessed Feb 2018
- Tisza MJ, Yuan H, Richard S, Buck CB (2016) Canine Papillomavirus 19 isolate tvmb1, complete genome. <https://www.ncbi.nlm.nih.gov/nucleotide/1064859043>. Accessed Feb 2018
- Tisza MJ, Yuan H, Schlegel R, Buck CB (2016) Genomic sequence of Canine Papillomavirus 19. *Genome Announc* 4 (6). <https://doi.org/10.1128/genomeA.01380-16>
- Lange CE, Diallo A, Zewe C, Ferrer L (2016) Novel canine papillomavirus type 18 found in pigmented plaques. *Papillomavirus Res* 2:159–163. <https://doi.org/10.1016/j.pvr.2016.08.001>
- Lange CE, Favrot C (2011) Canine papillomaviruses. *Vet Clin N Am Small Anim Pract* 41(6):1183–1195. <https://doi.org/10.1016/j.cvsm.2011.08.003>
- Munday JS, Dunowska M, Laurie RE, Hills S (2016) Genomic characterisation of canine papillomavirus type 17, a possible rare cause of canine oral squamous cell carcinoma. *Vet Microbiol* 182:135–140. <https://doi.org/10.1016/j.vetmic.2015.11.015>

20. Lange CE, Ackermann M, Favrot C, Tobler K (2012) Entire genomic sequence of novel canine papillomavirus type 13. *J Virol* 86(18):10226–10227. <https://doi.org/10.1128/JVI.01553-12>
21. Victoria JG, Kapoor A, Li L, Blinkova O, Slikas B, Wang C, Naeem A, Zaidi S, Delwart E (2009) Metagenomic analyses of viruses in stool samples from children with acute flaccid paralysis. *J Virol* 83(9):4642–4651. <https://doi.org/10.1128/JVI.02301-08>
22. Li L, Deng X, Mee ET, Collet-Teixeira S, Anderson R, Schepelmann S, Minor PD, Delwart E (2015) Comparing viral metagenomics methods using a highly multiplexed human viral pathogens reagent. *J Virol Methods* 213:139–146. <https://doi.org/10.1016/j.jviromet.2014.12.002>
23. Deng X, Naccache SN, Ng T, Federman S, Li L, Chiu CY, Delwart EL (2015) An ensemble strategy that significantly improves de novo assembly of microbial genomes from metagenomic next-generation sequencing data. *Nucleic Acids Res*. <https://doi.org/10.1093/nar/gkv002>
24. Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* 30(4):772–780. <https://doi.org/10.1093/molbev/mst010>
25. Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res* 30(14):3059–3066
26. Nei M, Kumar S (2000) *Molecular evolution and phylogenetics*. Oxford University Press, New York
27. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 30(12):2725–2729. <https://doi.org/10.1093/molbev/mst197>
28. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4(4):406–425. <https://doi.org/10.1093/oxfordjournals.molbev.a040454>
29. Tamura K, Nei M, Kumar S (2004) Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proc Natl Acad Sci USA* 101(30):11030–11035. <https://doi.org/10.1073/pnas.0404206101>
30. Thomas M, Myers MP, Massimi P, Guarnaccia C, Banks L (2016) Analysis of multiple HPV E6 PDZ interactions defines type-specific PDZ fingerprints that predict oncogenic potential. *PLoS Pathog* 12(8):e1005766. <https://doi.org/10.1371/journal.ppat.1005766>
31. Wang J, Zhou D, Prabhu A, Schlegel R, Yuan H (2010) The canine papillomavirus and gamma HPV E7 proteins use an alternative domain to bind and destabilize the retinoblastoma protein. *PLoS Pathog* 6(9):e1001089. <https://doi.org/10.1371/journal.ppat.1001089>
32. Schinazi RF, Goudgaon NM, Fulcrand G, el Kattan Y, Lesnikowski Z, Ullas G, Moravek J, Liotta DC (1994) Cellular pharmacology and biological activity of 5-carboranyl-2'-deoxyuridine. *Int J Radiat Oncol Biol Phys* 28(5):1113–1120
33. Bernard HU, Burk RD, Chen Z, van Doorslaer K, zur Hausen H, de Villiers EM (2010) Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. *Virology* 401(1):70–79. <https://doi.org/10.1016/j.virol.2010.02.002>
34. de Villiers E-M, Fauquet C, Broker TR, Bernard H-U, zur Hausen H (2004) Classification of papillomaviruses. *Virology* 324(1):17–27. <https://doi.org/10.1016/j.virol.2004.03.033>
35. Van Doorslaer K, Chen Z, Bernard HU, Chan PKS, DeSalle R, Dillner J, Forslund O, Haga T, McBride AA, Villa LL, Burk RD, Ictv Report C (2018) ICTV virus taxonomy profile: Papillomaviridae. *J Gen Virol* 99(8):989–990. <https://doi.org/10.1099/jgv.0.001105>
36. Munday JS, Dunowska M, Hills SF, Laurie RE (2013) Genomic characterization of *Felis catus* papillomavirus-3: a novel papillomavirus detected in a feline Bowenoid in situ carcinoma. *Vet Microbiol* 165(3–4):319–325. <https://doi.org/10.1016/j.vetmic.2013.04.006>
37. Dunowska M, Munday JS, Laurie RE, Hills SF (2014) Genomic characterisation of *Felis catus* papillomavirus 4, a novel papillomavirus detected in the oral cavity of a domestic cat. *Virus Genes* 48(1):111–119. <https://doi.org/10.1007/s11262-013-1002-3>
38. ICTV Report C (2017) Virus taxonomy: 2017 release Papillomaviridae. ICTV. https://talk.ictvonline.org/ictv-reports/ictv_9th_report/dsdna-viruses-2011/w/dsdna_viruses/121/papillomaviridae. 2018
39. Smits SL, Raj VS, Oduber MD, Schapendonk CM, Bodewes R, Provacia L, Stittelaar KJ, Osterhaus AD, Haagmans BL (2013) Metagenomic analysis of the ferret fecal viral flora. *PLoS ONE* 8(8):e71595. <https://doi.org/10.1371/journal.pone.0071595>
40. Priestnall SL, Mitchell JA, Walker CA, Erles K, Brownlie J (2014) New and emerging pathogens in canine infectious respiratory disease. *Vet Pathol* 51(2):492–504. <https://doi.org/10.1177/0300985813511130>
41. Munday JS, Witham AI (2010) Frequent detection of papillomavirus DNA in clinically normal skin of cats infected and noninfected with feline immunodeficiency virus. *Vet Dermatol* 21(3):307–310. <https://doi.org/10.1111/j.1365-3164.2009.00811.x>
42. Lange CE, Zollinger S, Tobler K, Ackermann M, Favrot C (2011) Clinically healthy skin of dogs is a potential reservoir for canine papillomaviruses. *J Clin Microbiol* 49(2):707–709. <https://doi.org/10.1128/JCM.02047-10>
43. Joffe DJ, Lelewski R, Weese JS, McGill-Worsley J, Shankel C, Mendonca S, Sager T, Smith M, Poljak Z (2016) Factors associated with development of Canine Infectious Respiratory Disease Complex (CIRDC) in dogs in 5 Canadian small animal clinics. *Can Vet J* 57(1):46–51

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.