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1 **Identification of canine papilloma virus by PCR in Greyhound dogs**

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17 Abstract

18 Background – Corns are hard protuberances that occur on the digital footpads of Greyhound
19 dogs. The cause of these lesions is unknown and there is little information about them in the
20 veterinary literature. We received anecdotal examples of dog to dog spread of corns suggesting
21 an infectious cause. The aim of this study was to determine if papillomavirus (PV) is associated
22 with Greyhound corns.

23

24 Methods – We examined four corns from two unrelated adult Greyhound dogs that resided in
25 Florida and Washington, respectively, for PV by PCR. The samples were obtained by owner
26 coring of two lesions from one dog and laser removal of two lesions from the other dog.

27 Total nucleic acid was extracted and DNA was amplified using two PCR primer sets that have
28 been shown to amplify a broad range of PVs from humans and animals: FAP59/ FAP64 and
29 MY11/ MY09. The DNA sequences were compared with all sequences in GenBank. Formalin-
30 fixed, paraffin-embedded tissue from the footpads of four dogs with other inflammatory
31 dermatoses were also examined.

32

33 Results – PV DNA was amplified from all four corn lesions, while no PV DNA was amplified
34 from other tissues. Comparison of the 300-400-bp sequences amplified by the MY11/ MY09
35 primers identified two different PVs. One showed 96% nucleotide sequence homology with the
36 L1 gene of canine PV type 12. The other showed 78% homology to canine PV type 16, and,
37 therefore, represents a novel PV. In one of the corns, infection by two of the identified PVs was
38 found.

39

40 Discussion – These results suggest PV infection could be involved in the pathogenesis of corns
41 in Greyhound dogs.

42

43

44 Introduction

45

46 Footpad lesions referred to as corns or paw pad keratomas, are hard protuberances that occur on
47 the digital footpads and seem to primarily affect Greyhound dogs. These lesions can be painful
48 and may be associated with lameness and poor performance (Gross et al, 2005). They are mostly
49 seen in middle-aged to older racing or retired racing Greyhound dogs (Balara et al, 2009;
50 Guilliard, Segboer and Shearer, 2010). The majority of corns occur in the center of the more
51 weight bearing digital pads of the front and/or hind feet but can also be found on the metacarpal
52 or metatarsal pads. Diagnosis of corns is usually based on the clinical appearance of
53 circumscribed hyperkeratosis on the paw pad (Gross et al, 2005). The cause of these lesions is
54 unknown and there is very little information about them in the veterinary literature. Theories as
55 to their cause include chronic trauma or pressure, deficiencies in the fatty layer of the pad, scar
56 tissue, foreign bodies or papillomavirus (PV) infection (Guilliard, Segboer and Shearer, 2010).

57

58 Papillomaviruses are a group of small, nonenveloped, double-stranded DNA that are
59 epitheliotropic. These epitheliotropic viruses infect a wide range of birds and mammals,
60 including humans, and cause benign cutaneous and mucosal epithelial proliferations called
61 papillomas (Lancaster and Olson, 1982). The goal of this study was to determine if PV was
62 associated with corns from two Greyhound dogs.

63

64

65 Materials and methods

66 Samples

67 Corns were acquired from two Greyhound dogs. Dog 1, an 8 year old female spayed retired
68 racing Greyhound dog from Florida, had a 2-3 year history of corns on digit 3 of both front paw
69 pads (figure 1). She had no prior history of corns until 3 months following adoption into a home
70 with another Greyhound dog with corns. Nail trimming equipment that was also used to de-bulk
71 the corns was shared between dogs. One corn from the left and right front feet were provided by
72 the owner for PCR analysis following a routine coring procedure. The samples were processed
73 upon receipt. Dog 2, a 6.5 year old male castrated retired racing Greyhound dog from Florida
74 who has resided for the past two years with his adopted family in Washington as an only pet, had
75 a 1 month history of lameness prior to referral. Corns were present on digit 3 of both hind paw
76 pads. There was also a single corn on the central portion of the metatarsal pad of the left hind
77 limb. Two corns from the digital pads were surgically removed by CO₂ laser. The corns were
78 initially placed in formalin, then transferred to saline and mailed to us for PCR analysis. The
79 samples were processed upon receipt.

80

81 PCR analysis

82 Total nucleic acid was extracted from the corn lesions and FFPE tissue scrolls using a
83 commercial kit (DNeasy blood and tissue kit; Qiagen, Valencia, CA, USA) according to the
84 manufacturer's protocol. The DNA was amplified using two PCR primer sets, FAP59/ FAP64
85 (Forslund et al, 1999) and MY11/ MY09 (Lurchachaiwong et al, 2009), that have been shown to
86 amplify diverse papillomavirus types from various mammalian tissues. Positive controls for the
87 FAP59/64 primers were DNA extracted from a feline Bowenoid in situ carcinoma, while no
88 template DNA (water only) was added to the negative controls. No positive control was used for
89 reactions with MY11/MY09 primers. PCR mixtures contained 1.5 µL each of forward and

90 reverse primers (concentration: 5 μ M), 6.5 μ L of nuclease-free water, 12.5 μ L of Taq premix
91 (rTaq® Takara Bio, Otsu, Shiga, Japan), and 5 μ L of DNA template. The same reaction
92 conditions previously described for the FAP59/64 primers (Forslund et al, 1999) were used for
93 all primer sets. The PCR products were analyzed by electrophoresis in a 1.4% agarose gel
94 containing ethidium bromide. To sequence PCR products, primers were digested using ExoSAP-
95 IT (USB, Cleveland, OH, USA), according to the manufacturer's instructions. Samples were
96 sequenced at the University of Tennessee Molecular Biology Resource Facility using Sanger
97 sequencing with an ABI prism dye terminator cycle sequencing reaction kit (Perkin Elmer Inc,
98 Foster City, CA, USA) and a capillary electrophoresis instrument (ABI 373 DNA, Perkin Elmer
99 Inc, Foster City, CA, USA). The PCR products were compared to sequences from GenBank
100 using the basic local alignment search tool
101 (BLAST;<http://www.ncbi.nlm.nih.gov/blast/Blast.cgi>). Three PCR products from three lesions
102 were cloned using the TOPO TA Cloning kit (Invitrogen, Carlsbad, CA, USA). Five clones from
103 each PV-positive sample were isolated and sequenced.

104

105 To confirm the etiologic link of PV with corns, formalin-fixed, paraffin-embedded (FFPE) tissue
106 from the footpads of four other dogs with various inflammatory diseases including pemphigus
107 foliaceus, hepatocutaneous syndrome, split paw pad disease, and parakeratosis with bacterial
108 colonization were also examined. The quality of the extracted nucleic acid of all the control
109 samples was confirmed using a 4200 TapeStation instrument. The presence of sufficient DNA
110 for amplification was determined by routine canine GAPDH PCR. Furthermore, our laboratory
111 was able to successfully amplify PV DNA from FFPE samples in previous studies (Anis et al,
112 2010; Newkirk et al, 2014).

113

114

115 Results

116

117 Both Primer sets were able to amplify papillomavirus DNA from all four corn lesions, while no
118 PV DNA was amplified from the other examined tissues. Comparison of the 300-400 bp
119 sequences amplified by the MY11/ MY09 primers identified two different PVs. One PV
120 amplified (300bp) from both dogs had 96% nucleotide sequence homology with the L1 gene
121 nucleotide sequence of the recently reported canine papillomavirus (CPV) type 12 (GenBank
122 accession No. JQ754321). The other viral DNA was amplified (400pb) and cloned only from
123 Dog 1. It revealed the greatest similarity to CPV type 16 (GenBank accession No. KP099966)
124 with 78% homology, and, therefore, represents a putative novel PV (GenBank accession No.
125 KU569988). In one of the examined corn lesions from Dog 1 there was a double infection by the
126 two identified CPV.

127

128 Discussion

129

130 These results are the first evidence that Greyhound dog corns may be associated with PV.
131 Attempts to link the condition to PV in the past have been unsuccessful. Histologically, the
132 lesions are characterized by well-defined conical hyperkeratosis that project above the skin
133 surface with no evidence of viral cytopathology or inflammation (Gross et al, 2005). A previous
134 study in which immunohistochemistry and PCR were performed on paraffin embedded tissue
135 obtained from six Greyhound dogs with corns failed to identify any PV DNA (Balara et al,

136 2009). Though that study used the same primer set used in the current study, the type of samples
137 as well as the annealing temperature were different. The sensitivity and specificity of the PCR
138 may be affected by many factors such as type of sample, DNA extraction procedure, purity of the
139 sample DNA and PCR setting. In the current study an annealing temperature of 50°C was used
140 instead of 55°C. This lower annealing temperature can amplify a broader range of DNA
141 templates (Ishii and Fukui, 2001). Furthermore, in the previous study the immunohistochemistry
142 was done using a single monoclonal antibody directed against human papillomavirus L1 capsid
143 epitope (Balara et al, 2009). Although this antibody was able to detect various PV including
144 HPV-1, 6, 11, 16, 18, and 31, its reactivity with all types has not been determined. In addition,
145 L1 capsid proteins are not expressed in all papilloma associated lesions, explaining possible false
146 negative results (Yemelyanova et al, 2013).

147 Papillomaviruses are an established cause of skin disease in dogs. They are circular, double-
148 stranded DNA viruses with a genome of approximately 8 kb pairs. Papillomaviruses are
149 classified into genus, species, and type based on the nucleotide sequence of the L1 open reading
150 frame. The L1 gene is highly conserved, and a new putative PV type is considered when the L1
151 nucleotide sequence is at least 10% different from other PV types (De Villiers, 2004). Currently
152 16 types of CPVs have been identified.¹¹ Canine PV type 12, which was isolated from three of
153 the corns in the present study, has been isolated and sequenced from a solitary pigmented plaque
154 on a mixed breed bloodhound (Zhou et al, 2015).¹²

155
156 To support the etiologic link of PV with corns in the present study, FFPE tissue from the
157 footpads of four other dogs with various inflammatory diseases were also examined. No
158 papillomavirus DNA was amplified from these examined lesions. Detecting PV within a lesion,
159 however, does not prove a causal relationship. Further study is needed to strengthen the
160 etiological link of PV with corns by performing IHC and/or in situ hybridization to localize PV
161 protein or DNA in a section of the corn lesions. Also more corn lesions need to be examined for
162 the presence of PV.

163

164 Conclusions

165 These results suggest that PV infection could be related to the pathogenesis of corns in
166 Greyhound dogs. Understanding the cause of this disease may lead to a more successful
167 treatment outcome.

168

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174 **References:**

175

176 Anis EA, O'Neill SH, Newkirk KM, Brahmabhatt RA, Abd-Eldaim M, Frank LA, Kania SA.
177 2010. Molecular characterization of the L1 gene of papillomaviruses in epithelial lesions of cats
178 and comparative analysis with corresponding gene sequences of human and feline
179 papillomaviruses. American Journal of Veterinary Research 71:1457-62.
180 Doi:10.2460.71.12.1457.

- 181 Balara JS, McCarthy RJ, Kiupel M, Buote MA, Wise AG, Maes RK. 2009. Clinical, histologic,
182 and immunohistochemical characterization of wart-like lesions on the paw pads of dogs: 24
183 cases (2000-2007). *Journal of the American Veterinary Medical Association* 234:1555-1558. doi:
184 10.2460/javma.234.12.1555.
- 185
- 186 De Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H. 2004. Classification of
187 papillomaviruses. *Virology* 324:17-27.
- 188
- 189 Forslund O, Antonsson A, Nordin P, Stenquist B, Hansson BG. 1999. A broad range of human
190 papillomavirus types detected with a general PCR method suitable for analysis of cutaneous
191 tumors and normal skin. *Journal of General Virology* 80:2437-2443.
- 192
- 193 Gross TL, Ihrke PJ, Walder EJ, et al. 2005. Epidermal tumours. In: Gross TL, Ihrke PJ, Walder
194 EJ, Affolter VK, eds. *Skin Diseases of the Dog and Cat*. 2nd edition. Oxford, UK: Blackwell
195 Publishing Ltd, 562-564.
- 196
- 197 Guilliard MJ, Segboer I, Shearer DH. 2010. Corns in dogs; signalment, possible aetiology and
198 response to surgical treatment. *Journal of Small Animal Practice* 51:162-168. doi:
199 10.1111/j.1748-5827.2010.00892.x.
- 200
- 201 Ishii K and Fukui M. 2001. Optimization of Annealing Temperature to reduce bias caused by a
202 primer mismatch in multitemplate PCR. *Applied and Environmental Microbiology* 67:3753-3755.
- 203
- 204 Lancaster WD, Olson C. 1982. Animal papillomaviruses. *Microbiological Reviews* 46:191-207.
- 205
- 206 Luff J, Mader M, Britton M, Fass J, Rowland P, Orr C, Schlegel R, Yuan H. 2015. Complete
207 genome sequence of canine papillomavirus type 16. *Genome Announcements* 7;3: pii: e00404-
208 15. doi: 10.1128/genomeA.00404-15.
- 209
- 210 Lurchachaiwong W, Junyangdikul P, Payungporn S, Chansaenroj J, Sampatanukul P, Tresukosol
211 D, Termrungruanglert W, Poovorawan Y. 2009. Relationship between hybrid capture II ratios
212 and DNA amplification of E1, E6 and L1 genes used for the detection of human papillomavirus
213 in samples with different cytological findings. *Asian Pacific Journal of Allergy Immunology* 27:
214 217-224.
- 215
- 216 Newkirk KM, Hendrix DVH, Anis EA, Rohrbach BW, Ehrhart EJ, Lyons JA, Kania SA. 2014.
217 Detection of papillomavirus in equine periocular and penile squamous cell carcinoma. *Journal of*
218 *Veterinary Diagnostic Investigation* 26:131-5. doi: 10.1177/1040638713511618.
- 219
- 220 Yemelyanova A, Gravitt PE, Ronnett BM, Rositch AF, Ogurtsova A, Seidman J, Roden
221 RB. 2013. Immunohistochemical detection of human papillomavirus capsid proteins L1 and L2
222 in squamous intraepithelial lesions: potential utility in diagnosis and management *Modern*
223 *Pathology* 26: 268-274. doi: 10.1038/modpathol.2012.156.
- 224

225 Zhou D, Luff J, Pau S, Alkhilaiwi F, Usuda Y, Wang N, Affolter V, Moore P, Schlegel R, Yuan
226 H. 2015. Complete genome sequence of canine papillomavirus virus type 12. Genome
227 Announcements 3: e00294-15. doi: 10.1128/genomeA.00294-15.

228

229 Figure Legend

230

231 Figure 1: Corn (arrow) on the left front digital pad of digit 3 from Dog 1.

232

233

234

235

Figure 1

Corn (arrow) on the left front digital pad of digit 3 from Dog 1.

