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Comparison of prevalence of *Felis catus* papillomavirus type 2 in squamous cell carcinomas in cats between Taiwan and Japan

Nanako YAMASHITA-KAWANISHI[1)], Chia Yu CHANG[2)], James K CHAMBERS[3)], Kazuyuki UCHIDA[3)], Katsuaki SUGIURA[4)], Iwao KUKIMOTO[5)], Hui Wen CHANG[2)] and Takeshi HAGA[1)]*

[1)]Division of Infection Control and Disease Prevention, Department of Veterinary Medical Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo 113-8657, Japan
[2)]Graduate Institute of Molecular and Comparative Pathobiology, School of Veterinary Medicine, National Taiwan University, Taipei 10617, Taiwan
[3)]Laboratory of Veterinary Pathology, Department of Veterinary Medical Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo 113-8657, Japan
[4)]Department of Global Agricultural Sciences, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo 113-8657, Japan
[5)]Pathogen Genomics Center, National Institute of Infectious Diseases, 4-7-1 Gakuen, Musashi-murayama, Tokyo 162-0052, Japan

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ABSTRACT. *Felis catus* papillomavirus (FcaPV), especially type 2 (FcaPV2) is considered as one of the causative agents in squamous cell carcinoma (SCC) in cats. However, our previous study detected FcaPV3 and FcaPV4, but not FcaPV2 in feline SCCs collected in Japan, suggesting that the prevalence of FcaPV2 in SCC may vary depending on geographic locations. To evaluate this hypothesis, two conventional PCR reactions targeting E1 and E7 genes were performed to detect FcaPV2 in feline SCC samples collected in Taiwan and Japan. While 46.9% (23/49) of feline SCC cases from Taiwan were PCR positive for FcaPV2, only 8.6% (3/35) cases from Japan were positive. Our result suggests that the prevalence of FcaPV2 in feline SCCs may depend on the region.

KEY WORDS: *Felis catus* papillomavirus type 2, Japan, molecular epidemiology, squamous cell carcinoma, Taiwan

Squamous cell carcinoma (SCC) is one of the common neoplastic diseases in cats, sharing about 15% of cutaneous tumors and the most in oral neoplasms [18, 27]. As one of the causative agents in feline SCC development, *Felis catus* papillomavirus (FcaPV) infection has been suggested [22, 24]. To date, six genotypes of FcaPVs have been detected and characterized from cutaneous/mucosal lesions in cats [8]. Among the six FcaPV types, FcaPV type 2 (FcaPV2) has been commonly identified in SCCs from the studies in Europe [2], Oceania [21, 29], and North America [26]. However, to our knowledge, no epidemiological information on FcaPV2 in Asian countries is available. In our previous study, FcaPV3 and FcaPV4, but not FcaPV2 was detected in feline SCC lesions collected in Japan [32], suggesting that distribution of FcaPV type may vary in different geographic regions worldwide. In cats, papillomaviral studies on viral plaques and Bowenoid *in situ* carcinomas (BISCs) are reported, as these diseases have potential to progress into SCCs [24, 26]. As well as in SCCs, FcaPV2 is considered to be the most prevalent genotype in viral plaques and BISCs in Europe and Oceania [1, 16, 30]. Based on these studies describing FcaPV2 detection at high rates in feline PV-associated neoplasms, a recent vaccinology study has focused on developing a viral-like particle (VLP) vaccine targeting FcaPV2 [28]. Although FcaPV2 is currently considered as a candidate FcaPV genotype for the first PV vaccine in cats [28], the information on FcaPV2-prevalence in Asian countries is scarce.

In humans, the divergence in high-risk human PV (HPV) type prevalence between geographical locations [10, 13] has been one of the considerable issues in vaccine development [9]. Similarly, to facilitate the development of a universally applicable and effective animal vaccine, molecular epidemiological studies are essential. Based on these backgrounds, this study aimed to identify the prevalence of FcaPV2 in SCC lesions of cats kept in Taiwan and Japan.

A total of 109 formalin-fixed paraffin-embedded (FFPE) tissue samples histologically diagnosed as feline SCC collected in

*Correspondence to: Haga, T.: ahaga@g.ecc.u-tokyo.ac.jp
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Taiwan (n=73) and Japan (n=36) [32] were analyzed. All of the samples from Taiwan and Japan were biopsy samples subjected for the purpose of histopathological diagnosis conducted at the Graduate Institute of Molecular and Comparative Pathobiology, School of Veterinary Medicine, National Taiwan University and the Laboratory of Veterinary Pathology, Department of Veterinary Medical Science, The University of Tokyo, respectively. Information of breed, sex, age, and lesion site of each case is summarized in Supplementary Table 1 and Supplementary Fig. 1. Overall, the mean age was 12.0 (Taiwan: 11.8; Japan: 12.2) years, ranged from 0.5 to 18. In both Taiwan- and Japan-derived samples, more female than male cats including the neutered animals were observed (Supplementary Fig. 1). The mean age of SCC-bearing cats was similar to that of the previous surveillance data (12.2 years), but SCC development was described to be more common in male than female cats in a previous study [11]. SCCs developed on the cutaneous sites were more common in Japanese cats (61%) than Taiwanese cats (40%) (Supplementary Fig. 1). Oral cavity such as the tongue and gingiva were common lesion site of the SCCs derived from the mucosal sites in both countries (Supplementary Table 1). Three cases from Japan were positive for FcaPV3 (sample ID: 13-0153) and FcaPV4 (13-136 and 14-1110) as described in the previous study [32].

Genomic DNA from FFPE tissue blocks was extracted using QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. The quantity and quality of the extracted DNA samples were measured using NanoDrop Lite (Thermo Fisher Scientific, Waltham, MA, USA). Conventional PCR was performed to detect feline beta-actin and FcaPV2 DNA by using DNA polymerase KOD FX Neo (Toyobo, Otsu, Japan). Considering the DNA fragmentation due to formalin fixation [6], primers were designed to amplify short sequences (<200 bp) of each target (Supplementary Table 2). Each PCR mixture was prepared as follows: 20 µl 2×PCR Buffer for KOD FX Neo, 4 µl dNTPs (2 mM), 0.4 µl forward primer (10 µM), 0.4 µl reverse primer (10 µM), and 0.4 µl KOD FX Neo (1 U/µl). Template DNA was added between 30 and 40 ng per PCR reaction and distilled water was filled up to a total volume of 20 µl. Distilled water instead of the template DNA was applied for negative controls. Extracted DNA sample from a feline BISC case was applied as positive controls for FcaPV2 E1 1769 (F)/1925 (R) and FcaPV2 E7 956 (F)/1130 (R) primer pairs. PCR cycling condition referring to the manufacturer's instructions was as follows: pre-denaturation at 94°C for 2 min followed by 39 cycles of denaturation at 98°C for 10 sec, annealing at respective melting temperatures for 30 sec and extension at 68°C for 15 sec. PCR-amplified products were electrophoresed on 2% agarose gels, stained with GelRed, and the bands were visualized with UV exposure. PCRs for FcaPV2 E1 and for FcaPV2 E7 exhibited the same sensitivity; the detection limit was 10 copies of FcaPV2 DNA per reaction (data not shown).

PCR results are summarized in Table 1 and Supplementary Table 1. Forty-nine out of 73 (67.1%) and 35 out of 36 (97.2%) samples from Taiwan and Japan were positive for feline beta-actin, respectively. Because DNA of beta-actin-negative samples might be degraded, samples positive for feline beta-actin were compared for FcaPV2 prevalence between Taiwan and Japan. However, given that background clinical information (breed, sex, lesion site, age, etc.) would be valuable epidemiological data for clinical veterinary medicine studies on feline SCC [11], such information of beta-actin-negative SCC cases is also included in Supplementary Table 1. Among the beta-actin-positive samples from Taiwan, 20.4% (10/49 samples) were both positive for FcaPV2 E1 and E7 (E1/E7). Some cases were positive for either E1 (14.3%, 7/49) or E7 (12.2%, 6/49) and 53.1% (26/49) were FcaPV2 E1/E7-negative. Three samples collected in Japan were positive for E1 and/or E7 (8.6%, 3/35) and one sample (2.9%, 1/35) was positive for E1 only. Thirty-two samples were negative for FcaPV2 E1/E7 (91.4%, 32/35) (Table 1). FcaPV2 E1/E7 prevalence between Taiwan and Japan was tested for significant difference with Fisher's extract two-sided test. E1 and/or E7-positive ratio was significantly higher in Taiwan-derived feline SCCs than Japan-derived samples (P=0.000029). No significant differences were evident in FcaPV2 E1/E7 double-positive ratios between the two countries (P=0.067).

Some samples from both countries were positive for either E1 or E7 (Supplementary Table 1). In humans, it has been described that disruptions of HPV E1 and E2 promote oncogenesis by accelerating E6/E7 expression [17]. A molecular epidemiological study on HPV18 showed that some of the cervical specimen samples including SCC lesions were single-positive for either E2 or E6/E7 determined by conventional PCR [31]. In animals, a study on *Canis familiaris* PV type 16 described that partial sequence of E1 and E2/E4 were deleted due to viral integration into the host gene in a canine cutaneous SCC case [14]. To the authors' knowledge, no sequence deletions associated with viral genome integration in cats have been previously reported. Further studies investigating the association between viral kinetics and SCC pathogenesis are needed to understand the phenomenon of FcaPV2 E1 or E7 single-positive cases.

Here, we described FcaPV2 prevalence discrepancies in feline SCCs between two island countries in Asia, Taiwan and Japan. PCR detection targeting FcaPV2 E1/E7 revealed that SCC lesions collected in Taiwan harbored higher prevalence than samples derived from Japan. Previous studies describing FcaPV2 detection in SCC lesions have been reported in Europe, Oceania, and North America. From the previous studies reported in New Zealand, 54% (38/70) [24] to 85% (17/20) [22] of cutaneous SCCs were described to be positive for FcaPV2. In North America, FcaPV2 was identified in 23% (9/39) to 50% (9/18) of feline cutaneous SCC samples by PCR [12, 26]. From a recent study reported in Italy, 31% (10/32) of feline oral SCC was positive for FcaPV2 DNA [2]. Based on these studies, FcaPV2 prevalence of Taiwan-derived SCC samples showed a similar ratio (46.9%) to those reported from non-Asian countries [2, 12, 22, 24, 26] whereas, FcaPV2-positive ratio was lower in SCC samples collected in Japan (8.6%). These results suggest that FcaPV2-prevalence may be different between two countries, however, sampling biases and limitations, such as sample numbers, domestic geographical origin, and lesion sites need to be considered. Regarding the domestic geographical origin, samples from Taiwan were derived from cats kept all over Taiwan, but most of the cases were from the Northern West. Specimens collected in Japan were biopsies excised from cats owned mostly in the Tokyo area (Kanto), while at least five cases from Japan (ID: 13-136; 5741-2013; 5757; 6997-2015; 5693) were derived from the southern part of Japan (Kyushu).

Table 1. Total and background factor-based *Felis catus* papillomavirus 2 PCR results of analyzed samples from Taiwan and Japan

Taiwan						Japan					
	FcaPV2 PCR						FcaPV2 PCR				
	E1 single-positive	E7 single-positive	E1/E7 double-positive	E1 and/or E7-positive	Negative		E1 single-positive	E7 single-positive	E1/E7 double-positive	E1 and/or E7-positive	Negative
ACTB-positive samples (n=49)	14.3 (7/49)	12.2 (6/49)	20.4 (10/49)	46.9 (23/49)*	53.1 (26/49)	ACTB-positive samples (n=35)	2.9 (1/35)	0 (0/35)	5.7 (2/35)	8.6 (3/35)*	91.4 (32/35)
Background factors											
Age						Age					
<5 (n=2)	0 (0/2)	0 (0/2)	50.0 (1/2)	50.0 (1/2)	50.0 (1/2)	<5 (n=0)	-	-	-	-	-
5–10 (n=12)	25.0 (3/12)	0 (0/12)	25.0 (3/12)	50.0 (6/12)	50.0 (6/12)	5–10 (n=9)	0 (0/9)	0 (0/9)	11.1 (1/9)	11.1 (1/9)	88.9 (8/9)
>10 (n=32)	9.4 (3/32)	15.6 (5/32)	18.8 (6/32)	43.8 (14/32)	56.3 (18/32)	>10 (n=26)	3.8 (1/26)	0 (0/26)	3.8 (1/26)	7.7 (2/26)	92.3 (24/26)
Unknown (n=3)	33.3 (1/3)	33.3 (1/3)	0 (0/3)	66.7 (2/3)	33.3 (1/3)						
Sex						Sex					
M (n=3)	0 (0/3)	33.3 (1/3)	0 (0/3)	33.3 (1/3)	66.7 (2/3)	M (n=7)	0 (0/7)	0 (0/7)	14.3 (1/7)	14.3 (1/7)	71.4 (5/7)
MX (n=21)	4.8 (1/21)	19.0 (4/21)	19.0 (4/21)	42.3 (9/21)	57.1 (12/21)	MX (n=9)	0 (0/9)	0 (0/9)	0 (0/9)	0 (0/9)	100 (9/9)
F (n=8)	12.5 (1/8)	0 (0/8)	25.0 (2/8)	37.5 (3/8)	62.5 (5/8)	F (n=4)	0 (0/4)	0 (0/4)	0 (0/4)	0 (0/4)	100 (4/4)
FX (n=15)	66.7 (4/15)	6.7 (1/15)	26.7 (4/15)	60.0 (9/15)	40.0 (6/15)	FX (n=15)	5.9 (1/15)	0 (0/15)	6.7 (1/15)	13.3 (2/15)	86.7 (13/15)
Unknown (n=2)	50.0 (1/2)	0 (0/2)	50.0 (1/2)	100 (2/2)	0 (0/2)						
Lesion site						Lesion site					
Cutaneous (n=19)	10.5 (2/19)	5.3 (1/19)	21.1 (4/19)	36.8 (7/19)	63.2 (12/19)	Cutaneous (n=21)	4.8 (1/21)	0 (0/21)	4.8 (1/21)	9.2 (2/21)	90.5 (19/21)
Mucosal (Oral) (n=19)	15.8 (3/19)	21.1 (4/19)	21.1 (4/19)	57.9 (11/19)	42.1 (8/19)	Mucosal (Oral) (n=9)	0 (0/9)	0 (0/9)	11.1 (1/9)	11.1 (1/9)	88.9 (8/9)
Mucosal (Non-oral) (n=10)	20.0 (2/10)	10.0 (1/10)	20.0 (2/10)	50.0 (5/10)	50.0 (5/10)	Mucosal (Non-oral) (n=5)	0 (0/5)	0 (0/5)	0 (0/5)	0 (0/5)	100 (5/5)
Unknown (n=1)	0 (0/1)	0 (0/1)	0 (0/1)	0 (0/1)	100 (1/1)						

PCR-positive ratio (%) and sample numbers of Taiwan- and Japan-derived SCC lesions are shown. Details and PCR results of each case are listed in Supplementary Table 1. ACTB, beta-actin; F, female; FX, neutered female; M, male; MX, neutered male. *Statistically significant difference between Taiwan and Japan ($P<0.01$), Fisher's extract two-sided test.

In cats, oral cavity and the skins of non-haired, non-pigmented areas of the body (pinna, nasal planum and eyelids) are common developing sites of SCC [7, 20, 25]. In this study, 37 out of 56 (66.1%) mucosal SCCs derived from both countries were lesions of the oral cavity including the tongue and gingiva (Supplementary Table 1). In humans, about a quarter of oral SCCs was described to have an association with PV infection [15]. Whereas in felids, detection ratio of PVs in oral SCCs differs between each study ranged from 0% (0/30) [23] to 31.3% (10/32) [2]. Here, 42.9% (12/28) of beta-actin-positive oral SCC samples derived from Taiwan (n=19) and Japan (n=9) were positive for FcaPV2 E1 and/or E7. Taiwan-derived oral SCC samples harbored higher FcaPV2 prevalence (57.9%, 11/19) than that of Japan-derived lesions (11.1%, 1/9), suggesting that PV prevalence may be divergent between lesion sites in addition to the country of origin. Although previous studies demonstrate the presence and gene expressions of FcaPV2 in feline oral SCCs [3, 4], additional evaluations are needed to clarify the significance of FcaPV2 infection and oral SCC development in cats.

Detection methodologies are one of the important factors in interpreting and conducting molecular epidemiological studies. The use of FFPE tissue blocks is a valuable source of DNA for retrospective researches on PV epidemiology. In our previous study, none of the analyzed SCC samples in Japan was positive for FcaPV2 by applying FcaPV2 type-specific primers designed to amplify a product of 419bp [32]. Likewise, none of the Taiwan-derived SCC samples included in this study was positive for 419 bp sequence of FcaPV2 using the same type-specific primer pair [32] (data not shown). In the present study, by applying the primers designed to target short sequences below 200 bp considering the DNA fragmentation [6], one sample (13-882) from our previous study [32] turned out to be positive for FcaPV2 E1/E7 (Supplementary Table 1). Similarly, previous literature described that primers targeting shorter sequences detected more PV DNA in FFPE-derived DNA samples [5], suggesting that primers amplifying short sequences may be desirable for performing PCR with FFPE-derived DNA samples.

There are two limitations in our study. First, the etiological roles of FcaPV2 in SCC development need to be investigated. In cats, as positive immunoreactivity of p16INK4a protein is considered as one of the observations indicative of PV infection and SCC development [3, 19, 32], immunohistochemical analysis targeting p16INK4a is preferable for proving the active involvement of FcaPV2 in the samples analyzed in this study. However, since the Taiwanese samples were available only as extracted DNA and some of the FFPE tissue blocks of the Japanese samples including three FcaPV2-positive cases (ID: 5757; 13-882; 16-0593) had run out, such immunohistochemical analysis was impossible to carry out. Both molecular and pathological analyses are thus essential for future studies to strengthen the association of PV infection with disease development. Another limitation was that unlike the Japan-derived SCC specimens [32], FcaPV types 3 and 4 were not investigated in the Taiwan-derived SCC specimens because some of the samples had been used up, precluding statistical comparison of FcaPV3/4 prevalence between the two countries. Further examination on various FcaPV types in larger sample sizes is required to better understand the molecular epidemiology of FcaPVs.

In conclusion, this study suggests that FcaPV2-prevalence in feline SCCs may be divergent across geographical areas. Further studies are required with larger sample sizes from various geographic origins including other Asian countries to enrich the knowledge on distributions and prevalence of FcaPV2. The present study describing the first molecular epidemiological report of FcaPV2 in Taiwan and Japan will contribute to the establishment of PV-associated disease prevention and treatment strategies.

CONFLICT OF INTEREST. The authors declare that they have no conflict of interest.

REFERENCES

1. Altamura, G., Jebara, G., Cardeti, G. and Borzacchiello, G. 2018. Felis catus papillomavirus type-2 but not type-1 is detectable and transcriptionally active in the blood of healthy cats. *Transbound. Emerg. Dis.* **65**: 497–503. [Medline] [CrossRef]
2. Altamura, G., Cardeti, G., Cersini, A., Eleni, C., Cocumelli, C., Bartolomé del Pino, L. E., Razzuoli, E., Martano, M., Maiolino, P. and Borzacchiello, G. 2020. Detection of Felis catus papillomavirus type-2 DNA and viral gene expression suggest active infection in feline oral squamous cell carcinoma. *Vet. Comp. Oncol.* vco.12569.
3. Altamura, G., Corteggio, A., Pacini, L., Conte, A., Pierantoni, G. M., Tommasino, M., Accardi, R. and Borzacchiello, G. 2016. Transforming properties of Felis catus papillomavirus type 2 E6 and E7 putative oncogenes in vitro and their transcriptional activity in feline squamous cell carcinoma in vivo. *Virology* **496**: 1–8. [Medline] [CrossRef]
4. Altamura, G., Power, K., Martano, M., Degli Uberti, B., Galiero, G., De Luca, G., Maiolino, P. and Borzacchiello, G. 2018. Felis catus papillomavirus type-2 E6 binds to E6AP, promotes E6AP/p53 binding and enhances p53 proteasomal degradation. *Sci. Rep.* **8**: 17529. [Medline] [CrossRef]
5. Alvarez-Aldana, A., Martínez, J. W. and Sepúlveda-Arias, J. C. 2015. Comparison of five protocols to extract DNA from paraffin-embedded tissues for the detection of human papillomavirus. *Pathol. Res. Pract.* **211**: 150–155. [Medline] [CrossRef]
6. Arreaza, G., Qiu, P., Pang, L., Albright, A., Hong, L. Z., Marton, M. J. and Levitan, D. 2016. Pre-analytical considerations for successful next-generation sequencing (ngs): challenges and opportunities for formalin-fixed and paraffin-embedded tumor tissue (FFPE) samples. *Int. J. Mol. Sci.* **17**: 1579. [Medline] [CrossRef]
7. Bertone, E. R., Snyder, L. A. and Moore, A. S. 2003. Environmental and lifestyle risk factors for oral squamous cell carcinoma in domestic cats. *J. Vet. Intern. Med.* **17**: 557–562. [Medline] [CrossRef]
8. Carrai, M., Van Brussel, K., Shi, M., Li, C. X., Chang, W. S., Munday, J. S., Voss, K., McLuckie, A., Taylor, D., Laws, A., Holmes, E. C., Barrs, V. R. and Beatty, J. A. 2020. Identification of a novel papillomavirus associated with squamous cell carcinoma in a domestic cat. *Viruses* **12**: 124. [Medline] [CrossRef]
9. Cheng, L., Wang, Y. and Du, J. 2020. Human papillomavirus vaccines: an updated review. *Vaccines (Basel)* **8**: 1–15. [Medline]
10. Clifford, G., Franceschi, S., Diaz, M., Muñoz, N. and Villa, L. L. 2006. Chapter 3: HPV type-distribution in women with and without cervical neoplastic diseases. *Vaccine* **24** Suppl 3: S3, 26–34. [Medline] [CrossRef]

11. Ho, N. T., Smith, K. C. and Dobromylskyj, M. J. 2018. Retrospective study of more than 9000 feline cutaneous tumours in the UK: 2006-2013. *J. Feline Med. Surg.* **20**: 128–134. [Medline] [CrossRef]
12. Hoggard, N., Munday, J. S. and Luff, J. 2018. Localization of felis catus papillomavirus type 2 E6 and E7 RNA in feline cutaneous squamous cell carcinoma. *Vet. Pathol.* **55**: 409–416. [Medline] [CrossRef]
13. Li, N., Franceschi, S., Howell-Jones, R., Snijders, P. J. F. and Clifford, G. M. 2011. Human papillomavirus type distribution in 30,848 invasive cervical cancers worldwide: Variation by geographical region, histological type and year of publication. *Int. J. Cancer* **128**: 927–935. [Medline] [CrossRef]
14. Luff, J., Mader, M., Rowland, P., Britton, M., Fass, J. and Yuan, H. 2019. Viral genome integration of canine papillomavirus 16. *Papillomavirus Res.* **7**: 88–96. [Medline] [CrossRef]
15. Marur, S., D'Souza, G., Westra, W. H. and Forastiere, A. A. 2010. HPV-associated head and neck cancer: a virus-related cancer epidemic. *Lancet Oncol.* **11**: 781–789. [Medline] [CrossRef]
16. Mazzei, M., Forzan, M., Carlucci, V., Anfossi, A. G., Alberti, A., Albanese, F., Binanti, D., Millanta, F., Baroncini, L., Pirone, A. and Abramo, F. 2018. A study of multiple Felis catus papillomavirus types (1, 2, 3, 4) in cat skin lesions in Italy by quantitative PCR. *J. Feline Med. Surg.* **20**: 772–779. [Medline] [CrossRef]
17. McBride, A. A. and Warburton, A. 2017. The role of integration in oncogenic progression of HPV-associated cancers. *PLoS Pathog.* **13**: e1006211. [Medline] [CrossRef]
18. Miller, W., Griffin, C. and Karen, C. 2012. Muller and Kirk's Small Animal Dermatology, 7th ed., Saunders, Philadelphia.
19. Munday, J. S. and Aberdein, D. 2012. Loss of retinoblastoma protein, but not p53, is associated with the presence of papillomaviral DNA in feline viral plaques, Bowenoid in situ carcinomas, and squamous cell carcinomas. *Vet. Pathol.* **49**: 538–545. [Medline] [CrossRef]
20. Munday, J. S. 2014. Papillomaviruses in felids. *Vet. J.* **199**: 340–347. [Medline] [CrossRef]
21. Munday, J. S., Gwyther, S., Thomson, N. A. and Malik, R. 2017. Bilateral pre-auricular papillary squamous cell carcinomas associated with papillomavirus infection in a domestic cat. *Vet. Dermatol.* **28**: 232–e58. [Medline] [CrossRef]
22. Munday, J. S., Kiupel, M., French, A. F. and Howe, L. 2008. Amplification of papillomaviral DNA sequences from a high proportion of feline cutaneous in situ and invasive squamous cell carcinomas using a nested polymerase chain reaction. *Vet. Dermatol.* **19**: 259–263. [Medline] [CrossRef]
23. Munday, J. S., Knight, C. G. and French, A. F. 2011. Evaluation of feline oral squamous cell carcinomas for p16CDKN2A protein immunoreactivity and the presence of papillomaviral DNA. *Res. Vet. Sci.* **90**: 280–283. [Medline] [CrossRef]
24. Munday, J. S., Gibson, I. and French, A. F. 2011. Papillomaviral DNA and increased p16CDKN2A protein are frequently present within feline cutaneous squamous cell carcinomas in ultraviolet-protected skin. *Vet. Dermatol.* **22**: 360–366. [Medline] [CrossRef]
25. Munday, J. S., Sharp, C. R. and Beatty, J. A. 2019. Novel viruses: Update on the significance of papillomavirus infections in cats. *J. Feline Med. Surg.* **21**: 409–418. [Medline] [CrossRef]
26. O'Neill, S. H., Newkirk, K. M., Anis, E. A., Brahmbhatt, R., Frank, L. A. and Kania, S. A. 2011. Detection of human papillomavirus DNA in feline premalignant and invasive squamous cell carcinoma. *Vet. Dermatol.* **22**: 68–74. [Medline] [CrossRef]
27. Stebbins, K. E., Morse, C. C. and Goldschmidt, M. H. 1989. Feline oral neoplasia: a ten-year survey. *Vet. Pathol.* **26**: 121–128. [Medline] [CrossRef]
28. Thomson, N. A., Howe, L., Weidgraaf, K., Thomas, D. G., Young, V., Ward, V. K. and Munday, J. S. 2019. Felis catus papillomavirus type 2 virus-like particle vaccine is safe and immunogenic but does not reduce FcaPV-2 viral loads in adult cats. *Vet. Immunol. Immunopathol.* **213**: 109888. [Medline] [CrossRef]
29. Thomson, N. A., Munday, J. S. and Dittmer, K. E. 2016. Frequent detection of transcriptionally active Felis catus papillomavirus 2 in feline cutaneous squamous cell carcinomas. *J. Gen. Virol.* **97**: 1189–1197. [Medline] [CrossRef]
30. Vascellari, M., Mazzei, M., Zanardello, C., Melchiotti, E., Albanese, F., Forzan, M., Croce, M. F., Alberti, A. and Abramo, F. 2019. *Felis catus* Papillomavirus Types 1, 2, 3, 4, and 5 in Feline Bowenoid in Situ Carcinoma: An In Situ Hybridization Study. *Vet. Pathol.* **56**: 818–825. [Medline] [CrossRef]
31. Yamaguchi-Naka, M., Onuki, M., Tenjimbayashi, Y., Hirose, Y., Tasaka, N., Satoh, T., Morisada, T., Iwata, T., Sekizawa, A., Matsumoto, K. and Kukimoto, I. 2020. Molecular epidemiology of human papillomavirus 18 infections in Japanese Women. *Infect. Genet. Evol.* **83**: 104345. [Medline] [CrossRef]
32. Yamashita-Kawanishi, N., Sawanobori, R., Matsumiya, K., Uema, A., Chambers, J. K., Uchida, K., Shimakura, H., Tsuzuki, M., Chang, C. Y., Chang, H. W. and Haga, T. 2018. Detection of felis catus papillomavirus type 3 and 4 DNA from squamous cell carcinoma cases of cats in Japan. *J. Vet. Med. Sci.* **80**: 1236–1240. [Medline] [CrossRef]