Veterinary Microbiology 238 (2019) 108426

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Prevalence and risk factors for *Felis catus gammaherpesvirus 1* detection in domestic cats in Italy

Francesca Caringella[a], Costantina Desario[a], Eleonora Lorusso[a], Ivana Pallante[a], Tommaso Furlanello[b], Gianvito Lanave[a], Gabriella Elia[a], Vito Martella[a], Roberta Iatta[a], Vanessa R. Barrs[c], Julia Beatty[c], Canio Buonavoglia[a], Nicola Decaro[a,*]

[a] Department of Veterinary Medicine, University of Bari, Valenzano, Bari, Italy
[b] San Marco Veterinary Clinic and Laboratory, Veggiano, Padova, Italy
[c] Faculty of Science, Sydney School of Veterinary Science, The University of Sydney, Sydney, NSW, 2006, Australia

ARTICLE INFO

Keywords:
Cats
Gammaherpesvirus
Molecular survey
Risk factors

ABSTRACT

Felis catus gammaherpesvirus 1 (FcaGHV1), a novel gammaherpesvirus of domestic cats identified in 2014, has been detected in different countries demonstrating a worldwide distribution. The aim of this study was to establish the prevalence of FcaGHV1 in Italy using a molecular epidemiological approach. FcaGHV1 DNA was detected with virus-specific real-time PCR in ≃1% of 2659 feline blood samples tested. Analysis of risk factors showed that being male and coinfection with feline immunodeficiency virus (FIV) increase the likelihood of FcaGHV1 detection. One-third of FcaGHV1-positive cats also tested positive for FIV provirus, whereas coinfections with feline panleukopenia virus were not demonstrated. Further studies are necessary to confirm the risk factors for FcaGHV1 detection and the pathobiology of the virus.

1. Introduction

Herpesviridae are double-stranded DNA viruses (130-220 kbp) that comprise three subfamilies (*Alpha-*, *Beta-*, and *Gammaherpesvirinae*) on the basis of genomic, serologic, biologic and morphologic criteria. The *Gammaherpesvirinae* subfamily, which includes four genera: *Lymphocryptovirus*, *Macavirus*, *Percavirus* and *Rhadinovirus* (Davison et al., 2009), is expanding as more members are discovered. Gammaherpesviruses (GHVs) are ubiquitous viruses of humans and other animals and they can replicate in T or B lymphocytes with subsequent latency in the lymphoid organs and tissues (McLuckie et al., 2016a).

For many years domestic cats have been considered natural hosts of a single herpesvirus, *Felid alphaherpesvirus 1* (FHV-1), which is responsible for ocular and respiratory disease (Gould, 2011). However, in 2014 a molecular survey carried out in the United States identified a new feline herpesvirus displaying a genetic similarity to gammaherpesviruses. This virus was referred to as *Felis catus gammaherpesvirus 1* (FcaGHV1) and is now included in the *Percavirus* genus based on nucleotide identity with the members of the same genus (Troyer et al., 2014). After its first discovery, FcaGHV1 was identified in different geographic areas such as Australia, Europe, Singapore, North America and Brazil (Beatty et al., 2014; Ertl et al., 2015; Makundi et al., 2018; McLuckie et al., 2016a,b; Kurissio et al., 2018; Troyer et al., 2014), with detection rates between 9.6 and 23.6%.

Risk factors for FcaGHV1 infection are reported to be age, sex, health status and coinfections with other microorganisms. A higher frequency of infection is found in male, adult and intact cats, which might reflect horizontal transmission during territorial aggression (Beatty et al., 2014; Troyer et al., 2014). Additional risk factors are represented by coinfections with retroviruses such as feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV). Immunocompromised individuals are more susceptible to develop cancer and lymphoproliferative disorders in association with GHVs, while in immunocompetent patients the infection usually remains subclinical (Means et al., 2007; Kaye et al., 2016; McLuckie et al., 2017). In humans, a high percentage of lymphomas induced by the interaction between human immunodeficiency virus (HIV) and some GHVs, including Epstein-Barr virus (EBV) and Kaposi's sarcoma–associated herpesvirus (KSHV), has been demonstrated (Heslop et al., 2005; Carbone et al., 2009). The pathobiological role of FcaGHV-1 in cats is not clear, although FcaGHV1 DNAemia has been associated with reduced survival in cats with high-grade lymphoma. (Beatty et al., 2019).

In Italy, the FcaGHV-1 detection has not been assessed in the general population so far, and a recent study failed to detect this GHV in

* Corresponding author at: Department of Veterinary Medicine, University of Bari, Strada Prov. per Casamassima km 3, 70010, Valenzano, Bari, Italy.
E-mail address: nicola.decaro@uniba.it (N. Decaro).

https://doi.org/10.1016/j.vetmic.2019.108426
Received 12 August 2019; Received in revised form 15 September 2019; Accepted 20 September 2019

feline blood donors (Marenzoni et al., 2018). Therefore, the aim of the present study was to assess the prevalence of FcaGHV-1 DNA detection in the Italian cat population and to identify potential risk factors.

2. Materials and methods

2.1. Sample collection

A total of 2659 EDTA-blood samples were collected from cats submitted during routine clinical examination in veterinary clinics from all over Italy. Specifically, 1543 samples came from northern Italy, 471 from central Italy and 645 from southern Italy. For each cat, data regarding age, sex, breed, reproductive status and location of domicile were collected. Blood samples for inclusion in this study were all submissions to clinical pathology laboratories for the period June 2017-August 2018. Sampled animals included both healthy animals tested for routine screening and sick animals with clinical signs. Data on the health status of individual animals within the population set was not available.

2.2. Ethics statement

The samples were recruited from a study, which had been performed to assess the prevalence of feline leishmaniasis in Italy by the Parasitology Unit of the Department of Veterinary Medicine of Bari (Iatta et al., 2019) and whose protocol had been approved by the Ethical Committee of the Department (authorization no. 7/17).

2.3. Sample preparation

DNA was extracted from blood samples using the commercial kit GenUP-DNA kit (BiotechRabbit, Germany) following the producer's instructions. The DNAs were stored at −20 °C for a maximum of 20 months prior to testing.

Samples were tested in pools of 5 samples each for multiple molecular investigations in order to evaluate coinfections of FcaGHV1 and other common viral pathogens of cats. All positive pools were then screened individually.

2.4. Molecular investigations

2.4.1. Real-time PCR for FcaGHV1

A real-time PCR (qPCR) protocol previously described was used to detect FcaGHV1 and quantify the viral DNA (Troyer et al., 2014). Primer pair FGHV-F3 (5′-ACATCTTCACTGGACAACTGG) and FGHV-R3 (5′-GTGCATTTGATGTCCTGACTG) was used with the probe FGHV-P3 (5′-TGAACAGCTGAGTCTCTACAAGTCTCCA), which targeted the gB gene. Ten microliters of DNA were added to 15 µl of mix, prepared with iTaq™ Universal Probes Supermix (Bio-Rad Laboratories Srl, Milan, Italy), 400 nM of each primer and 200 nM of probe, for a total volume of 25 µl. The thermal protocol for FcaGHV1 included a first step at 95 °C for 3 min, followed by 45 cycles of 95 °C for 5 s and 60 °C for 30 s. As positive control a plasmid was used, which was prepared by cloning the gB gene with the TOPO TA cloning kit (Life Technologies) following the manufacturer's instructions. Samples yielding cycle threshold values higher than 40 were retested to confirm the FcaGHV1 positive result and only samples testing positive on two consecutive assays were considered true positives.

2.4.2. Screening assays for FIV, FeLV and feline panleukopenia virus (FPLV)

The samples were also used for the detection of proviral DNAs of FIV and FeLV by conventional PCR using protocols previously described (Quackenbush et al., 1996; Stiles et al., 1999).

FPLV DNA was detected by means of real-time PCR assays. The first protocol with a TaqMan probe was able to recognize all members of the species *Carnivore protoparvovirus 1* (Decaro et al., 2005a), while minor grove binder probe assays were employed to discriminate between FPLV and canine parvovirus (CPV) and to characterize the CPV strains (Decaro et al., 2005b, 2006; Decaro et al., 2008).

2.5. Statistical analyses

The calculation of 95% confidence intervals (95% CI) for the age was carried out using the online available software Calculator of the Confidence Interval (http://www.learningaboutelectronics.com). The categorical variables related to age and sex factors were examined using the Chi-Square test, while coinfections of pathogens in the same sample were investigated by Fisher's exact test. Statistical analyses were performed using a statistical calculation software available online (Social Science Statistics, https://www.socscistatistics.com/) setting a statistical significance of $p < 0.05$. Odds Ratio's calculation (OR) along with the related CI 95% was performed using the software Select Statistical Services (https://select-statistics.co.uk/calculators/confidence-interval-calculator-odds-ratio/).

3. Results

A total of 27 samples tested positive for FcaGHV1, showing a detection rate of 1.01% (27/2659) (Table 1).

The analyzed samples were collected from different geographical areas in Italy: 58% (1543/2659) came from the North, 17.7% (471/2659) from the Center and 24.3% (645/2659) from southern Italy. Based on the geographic regions, the samples showed a prevalence of 0.9% (14/1543) for FcaGHV1 in northern Italy, 1.3% (6/471) in the Center, 1.1% (7/645) in southern Italy.

Regarding the sex of the cats examined, 1302 were males (48.96%) and 1357 females (51.03%). Among FcaGHV1-positive cats, 70.4% (19/27) were male and 29.6% (8/27) female. A statistically significant association between sex and FcaGHV1 positivity was identified ($p = 0.02$).

The age of the cats examined varied from 1 month to 256 months [mean 100 months, CI95% = (102.5; 107.5)]. Cats were divided into groups based on their age: i) juvenile cats < 18 months (13.5%, 359/2659), ii) adults aged between 19 months and 72 months (22.5%, 599/2659) and iii) elderly aged > 72 months (64%, 1701/2659). Of the FcaGHV1-positive cats, 11.1% (3/27) were included in young cats, 25.9% (7/27) in the adult group and 63% (17/27) in the elderly group. Age was not significantly associated with FcaGHV1-positivity ($p = 0.25$).

Regarding desexing status, 2275 cats were neutered (85.6%, 2275/2659) and 384 intact (14.4%, 384/2659); among the FcaGHV1 positives, almost all were neutered (88.9%, 24/27) and only 3 were entire (11.1%, 3/27).

As for other investigated pathogens, the prevalence of FeLV provirus detection was the highest (4.17%, 111/2659), followed by FIV provirus (3.84%, 102/2659) and then FPLV DNA (0.86%, 23/2659). Coinfections were identified in 24 samples analyzed, including 13 FIV/FeLV, 9 FcaGHV1/FIV, 1 FcaGHV1/FeLV, 1 FeLV/FPLV coinfection. In two cases, a triple (FcaGHV-1, FIV and FeLV) infection was observed (Fig. 1). There were no FcaGHV1/FPLV or FIV/FPLV coinfections.

A total of 55.6% (15/27) of the FcaGHV1-positive cats were infected by a single virus; the remaining 12 cats were coinfected either with FIV (33.3%, 9) or FeLV (3.7%, 1) or with both retroviruses (7.4%, 2). Among the coinfections, FIV was significantly associated with FcaGHV1 detection [$p = 0.00001$, OR = 13.65, CI95% = (5.97; 31.19)] while FeLV coinfection was not. In addition, coinfection with FIV and FeLV concurrently was significantly associated with FcaGHV1 detection [($p = 0.0072$, OR = 19.06, IC95% = (4.02; 90.46)].

The analysis related to the FIV/FeLV coinfection on the total of 2659 examined cats showed a statistical significance in comparison with single infections [$p = 0.0002$, OR = 3.67, IC95% = (1.98; 6.79)]

Table 1
Details of FcaGHV1 positive samples.

Sample No.	FcaGHV1 real-time PCR C_T values	FPLV	FIV/FeLV	Geographic are	Age (months)	Sex	Breed	Desexing status
1	27.49	Neg	Neg	Center	84	M	Maine coon	N
2	35.91	Neg	Neg	Center	174	F	European	N
3	39.08	Neg	FIV	Center	36	M	European	N
4	35.16	Neg	Neg	North	156	F	European	N
5	37.29	Neg	Neg	North	12	F	European	N
6	36.18	Neg	FIV, FeLV	North	84	M	European	I
7	35.31	Neg	FIV	North	180	M	European	N
8	33.77	Neg	Neg	North	197	F	European	N
9	37.10	Neg	Neg	North	122	M	European	N
10	33.45	Neg	FIV	North	180	F	European	N
11	34.66	Neg	Neg	South	24	M	European	N
12	36.88	Neg	Neg	North	156	F	European	N
13	34.04	Neg	Neg	North	168	M	European	N
14	41.88	Neg	Neg	North	138	F	European	N
15	31.29	Neg	Neg	North	107	M	European	N
16	42.72	Neg	Neg	North	177	M	European	N
17	29.80	Neg	Neg	Center	120	M	European	N
18	36.03	Neg	FIV	North	60	M	European	N
19	35.34	Neg	FIV	North	180	M	European	N
20	36.33	Neg	Neg	Center	144	F	European	N
21	35.95	Neg	FIV	Center	48	M	European	N
22	34.25	Neg	FIV	South	12	M	European	I
23	34.01	Neg	FIV	South	84	M	Siamese	N
24	34.81	Neg	FIV, FeLV	South	48	M	European	N
25	36.49	Neg	FIV	South	72	M	European	N
26	35.44	Neg	FeLV	South	24	M	European	N
27	40.57	Neg	Neg	South	12	M	European	I

Neg, negative; N, neutered; I; intact.

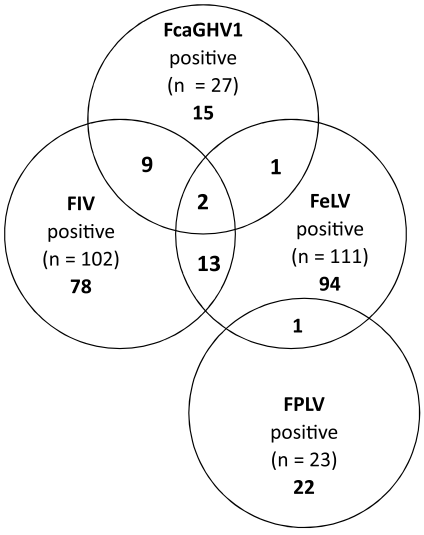

Fig. 1. Graphic representation of single infections and coinfections detected in this study. The total number of infections by each virus is presented in parentheses, while single infections and coinfections are represented by bolded numbers.

in contrast to the FeLV/FPLV coinfection, which had no statistical significance (Table 2).

Since the same samples had been tested for *Leishmania infantum* (Iatta et al., 2019), we searched for possible coinfections between this protozoon and FcaGHV1, detecting only 3 and 0 coinfections among the 104 and 22 samples that had tested leishmania positive by serology and real-time PCR, respectively. Accordingly, no statistical association was observed between FcaGHV1 and leishmania positive samples [p = 0.0844, OR = 3.15, CI95% = (0.94; 10.67)].

Table 2
Prevalence and statistical significance of coinfections.

FcaGHV1	***versus* FIV** C: 9 (0.3%), OR: 13.65, CI95%: [5.97; 31.19], p: 0.00001 ***	***versus* FeLV** C: 1 (0.04%), OR: 0.88, CI95%: [0.12; 6.56], p: > 0.05 N.S.	***versus* FPLV** NC	***versus* FIV and FeLV** C: 2 (0.08%) OR: 19.06, CI95%: [4.02; 90.46], p: 0.0072 **
FIV	***versus* FeLV** 13 (0.49%), OR: 3.67, CI95%: [1.98; 6.79], p: 0.0002 ***	***versus* FPLV** NC		
FeLV	***versus* FPLV** 1 (0.04%), OR: 1.04, CI95%: [0.14; 7.81], p: > 0.05 NS			

C, coinfection number and percentage (in parentheses) on the total samples (2659); OR, Odds ratio; CI95%, 95% confidence interval; p, p-value of Fisher's exact test; *, significant (0.01 < x < 0.05); **, very significant (0.001 < x < 0.01); ***, highly significant (x < 0.001); NC, Not coinfected; NS, Not significant.

4. Discussion

The present study represents the first large-scale epidemiological survey performed across Italy to assess the molecular prevalence of FcaGHV1 in domestic cats. Our study shows that only 1.01% of blood samples from cats in Italy tested FcaGHV-positive, which is much lower than observed in other geographical areas where prevalences of 16% and 23% are reported (Beatty et al., 2014; Ertl et al., 2015; Makundi et al., 2018; McLuckie et al., 2016b; Kurissio et al., 2018; Troyer et al., 2014). A previous study from Italy failed to detect FcaGHV1 in blood samples from 31 blood donor cats (Marenzoni et al., 2018), a result

which is not dissimilar to that of the current study.

A similar result was found in Japanese cats that showed a prevalence of FcaGHV1 around 1.3% (Tateno et al., 2017). However, the Japanese study used a degenerate panherpesvirus nested PCR approach, which is less sensitive than the FcaGHV1 specific real-time PCR assay, thus likely accounting for the apparent low prevalence observed in that study. Accordingly, a subsequent Japanese study (albeit on an island population) using a virus specific PCR found a prevalence similar to other regions (Makundi et al., 2018).

Our findings suggest that the molecular prevalence of FcaGHV1 is genuinely lower in Italy than in other regions investigated to date, although the reason why FcaGHV1 DNAemia is less common in Italian domestic cats should be clarified. Our study used the same real-time PCR protocol employed in studies showing higher virus prevalences (McLuckie et al., 2016b; Troyer et al., 2014), thus reducing concern related to the test sensitivity, also considering that the assays displayed a high sensitivity when testing ten-fold dilutions of the plasmid DNA. Theoretically, the long-term storage of the tested samples may have affected a successful detection of the virus in low-titre samples. However, it should be considered that the GHV DNA is double-stranded, thus accounting for a certain resistance to the degradation consequent to the storage in the freezer.

Our study showed a statistically significant association between being male and the positivity to FcaGHV1; male cats were at least twice as likely as females to be FcaGHV1 DNAemic. This result is consistent with previous studies reporting that males are more likely to have a FcaGHV1 DNAemia than female cats, perhaps due to the territorial fights (Beatty et al., 2014).

The majority of FcaGHV1 positive cats (63%, 17/27) belonged to the elderly category (cats older than 72 months) but age was not identified as a risk factor for a positive FcaGHV1 PCR result. This result may have been influenced by sampling bias, as 63.3% (1684/2659) of the tested animals were older than 72 months. Interestingly, in previous studies, advanced age was identified as a risk factor for FcaGHV1 detection (Beatty et al., 2014).

The higher rates of FcaGHV1 detection in the male rather than female populations and in the elderly rather than in the juvenile populations (this last data not confirmed in our study), may reflect horizontal transmission of the virus. Males are more likely involved in fights than females considering their territorial nature. Young cats participate less frequently in fighting activities and therefore they are less likely in contact with the virus. FcaGHV1 could share the same transmission pathway with other co-pathogens identified as risk factors, including FIV which is transmitted through bites and saliva inoculation (Yamamoto et al., 1989). In this context, our finding that leishmania coinfection is not a risk factor for FcaGHV1 DNAemia is not surprising, since leishmania is a vector-borne infection.

In the present study, the statistical correlation between FIV and FcaGHV1 found in previous studies was confirmed (Beatty et al., 2014). Accordingly, FIV-induced immunosuppression could be another of the predisposing factors for FcaGHV1 infection (Ertl et al., 2015). FIV-induced immunosuppression could determine a reactivation of FcaGHV1 from the latency phase and increase the viral load. This mirrors what occurs in HIV infections in humans that increase the viral load of the Epstein-Barr virus (Beatty et al., 2014). Data on another pathogenic feline retrovirus, FeLV, were available for cats from Singapore and showed that FeLV infection was significantly associated with FcaGHV1 detection (Beatty et al., 2014). However, FeLV coinfection was not significant in a subsequent study (McLuckie et al., 2017). In our study, an absence of statistical correlation between FeLV and FcaGHV1 coinfections was evident, along with a lack of FPLV and FcaGHV1 coinfections.

In conclusion, the present study shows that FcaGHV1 circulates in domestic cats in Italy supporting a likely worldwide distribution of the virus. Although FcaGHV1 is considered emerging, there is no clear association of the virus to clinical disease, so that it could be underestimated in clinical and laboratory diagnosis of patients undergoing medical examination. Therefore, more studies are needed in order to demonstrate or rule out a pathogenetic role for FcaGHV1 in cats.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Acknowledgments

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Beatty, J.A., Troyer, R.M., Carver, S., Barrs, V.R., Espinasse, F., Conradi, O., Stutzman-Rodriguez, K., Chan, C.C., Tasker, S., Lappin, M.R., VandeWoude, S., 2014. Felis catus gammaherpesvirus 1; a widely endemic potential pathogen of domestic cats. Virology 460-461, 100–107. https://doi.org/10.1016/j.virol.2014.05.007.

Beatty, J.A., Sharp, C.R., Duprex, W.P., Munday, J.S., 2019. Novel feline viruses: emerging significance of gammaherpesvirus and morbillivirus infections. J. Feline Med. Surg. 21, 5–11. https://doi.org/10.1177/1098612X18808102.

Carbone, A., Cesarman, E., Spina, M., Gloghini, A., Schulz, T.F., 2009. HIV-associated lymphomas and gamma-herpesviruses. Blood 113, 1213–1224. https://doi.org/10.1182/blood-2008-09-180315.

Davison, A.J., Eberle, R., Ehlers, B., Hayward, G.S., McGeoch, D.J., Minson, A.C., Pellett, P.E., Roizman, B., Studdert, M.J., Thiry, E., 2009. The order herpesvirales. Arch. Virol. 154, 171–177. https://doi.org/10.1007/s00705-008-0278-4.

Decaro, N., Desario, C., Campolo, M., Elia, G., Martella, V., Ricci, D., Lorusso, E., Buonavoglia, C., 2005a. Clinical and virological findings in pups naturally infected by canine parvovirus type 2 Glu-426 mutant. J. Vet. Diagn. Invest. 17, 133–138. https://doi.org/10.1177/104063870501700206.

Decaro, N., Elia, G., Campolo, M., Desario, C., Lucente, M.S., Bellacicco, A.L., Buonavoglia, C., 2005b. New approaches for the molecular characterization of canine parvovirus type 2 strains. J. Vet. Med. B Infect. Dis. Vet. Public Health 52, 316–319. https://doi.org/10.1111/j.1439-0450.2005.00869.x.

Decaro, N., Elia, G., Martella, V., Campolo, M., Desario, C., Camero, M., Cirone, F., Lorusso, E., Lucente, M.S., Narcisi, D., Scalia, P., Buonavoglia, C., 2006. Characterisation of the canine parvovirus type variants using minor groove binder probe technology. J. Virol. Methods 133, 92–99. https://doi.org/10.1016/j.jviromet.2005.10.026.

Decaro, N., Desario, C., Lucente, M.S., Amorisco, F., Campolo, M., Elia, G., Cavalli, A., Martella, V., Buonavoglia, C., 2008. Specific identification of feline panleukopenia virus and its rapid differentiation from canine parvoviruses using minor groove binder probes. J. Virol. Methods 147, 67–71. https://doi.org/10.1016/j.jviromet.2007.08.006.

Ertl, R., Korb, M., Langbein-Detsch, I., Klein, D., 2015. Prevalence and risk factors of gammaherpesvirus infection in domestic cats in Central Europe. Virol. J. 12, 146. https://doi.org/10.1186/s12985-015-0381-6.

Gould, D., 2011. Feline herpesvirus-1: ocular manifestations, diagnosis and treatment options. J. Feline Med. Surg. 13, 333–346. https://doi.org/10.1016/j.jfms.2011.03.010.

Heslop, H.E., 2005. Biology and treatment of Epstein-Barr virus–associated non-Hodgkin lymphomas. Hematol. Am. Soc. Hematol. Educ. Program 260–266. https://doi.org/10.1182/asheducation-2005.1.260.

Iatta, R., Furlanello, T., Colella, V., Tarallo, V.D., Latrofa, M.S., Brianti, E., Trerotoli, P., Decaro, N., Lorusso, E., Schunack, B., Mirò, G., Dantas-Torres, F., Otranto, D., 2019. A nationwide survey of Leishmania infantum infection in cats and associated risk factors in Italy. PLoS Negl. Trop. Dis. 13, e0007594. https://doi.org/10.1371/journal.pntd.0007594.

Kaye, S., Wang, W., Miller, C., McLuckie, A., Beatty, J.A., Grant, C.K., VandeWoude, S., Bielefeldt-Ohmann, H., 2016. Role of feline immunodeficiency virus in lymphomagenesis-going alone or colluding? ILAR J. 57, 24–33. https://doi.org/10.1093/ilar/ilv047.

Kurissio, J.K., Rodrigues, M.V., Taniwaki, S.A., de Souza Zanutto, M., Filoni, C., Galdino, M.C., Junior, J.P.A., 2018. Felis catus gammaherpesvirus 1 (FcaGHV1) and coinfections with feline viral pathogens in domestic cats in Brazil. Ciência Rural 48, 03. https://doi.org/10.1590/0103-8478cr20170480.

Makundi, I., Koshida, Y., Endo, Y., Nishigaki, K., 2018. Identification of Felis catus gammaherpesvirus 1 in Tsushima Leopard Cats (*Prionailurus bengalensis euptilurus*) on Tsushima Island, Japan. Viruses 10, E378. https://doi.org/10.3390/v10070378.

Marenzoni, M.L., Antognoni, M.T., Baldelli, F., Miglio, A., Stefanetti, V., Desario, C., Di Summa, A., Buonavoglia, C., Decaro, N., 2018. Detection of parvovirus and herpesvirus DNA in the blood of feline and canine blood donors. Vet. Microbiol. 224, 66–69. https://doi.org/10.1016/j.vetmic.2018.08.030.

McLuckie, A.J., Barrs, V.R., Smith, A.L., Beatty, J.A., 2016a. Detection of Felis catus gammaherpesvirus 1 (FcaGHV1) in peripheral blood B- and T-lymphocytes in asymptomatic, naturally-infected domestic cats. Virology 497, 211–216. https://doi.org/10.1016/j.virol.2016.07.018.

McLuckie, A.J., Tasker, S., Dhand, N.K., Spencer, S., Beatty, J.A., 2016b. High prevalence

of Felis catus gammaherpesvirus 1 infection in haemoplasma-infected cats supports co-transmission. Vet. J. 214, 117–121. https://doi.org/10.1016/j.tvjl.2016.06.001.

McLuckie, A.J., Barrs, V.R., Wilson, B., Westman, M.E., Beatty, J.A., 2017. Felis catus gammaherpesvirus 1 DNAemia in whole blood from therapeutically immunesuppressed or retrovirus-infected cats. Vet. Sci. 4, E16. https://doi.org/10.3390/vetsci4010016.

Means, R.E., Lang, S.M., Jung, J.U., 2007. Human gammaherpesvirus immune evasion strategies. Human Herpesviruses: Biology, Therapy and Immunoprophylaxis. Cambridge University Press Chapter 31.

Quackenbush, S.L., Dean, G.A., Mullins, J.I., Hoover, E.A., 1996. Analysis of FeLV-FAIDS provirus burden and productive infection in lymphocyte subsets in vivo. Virology 223, 1–9. https://doi.org/10.1006/viro.1996.0449.

Stiles, J., Bienzle, D., Render, J.A., Buyukmihci, N.C., Johnson, E.C., 1999. Use of nested polymerase chain reaction (PCR) for detection of retroviruses from formalin-fixed, paraffin-embedded uveal melanomas in cats. Vet. Ophthalmol. 2, 113–116.

Tateno, M., Takahashi, M., Miyake, E., Nishigaki, K., Tsujimoto, H., Endo, Y., 2017. Molecular epidemiological study of gammaherpesvirus in domestic cats in Japan. J. Vet. Med. Sci. 79, 1735–1740. https://doi.org/10.1292/jvms.17-0039.

Troyer, R.M., Beatty, J.A., Stutzman-Rodriguez, K.R., Carver, S., Lozano, C.C., Lee, J.S., Lappin, M.R., Riley, S.P.D., Serieys, L.E.K., Logan, K.A., Sweanor, L.L., Boyce, W.M., Vickers, T.W., McBride, R., Crooks, K.R., Lewis, J.S., Cunningham, M.W., Rovnak, J., Quackenbush, S.L., VandeWoude, S., 2014. Novel gammaherpesviruses in North American domestic cats, bobcats, and pumas: identification, prevalence, and risk factors. J. Virol. 88, 3914–3924. https://doi.org/10.1128/JVI.03405-13.

Yamamoto, J.K., Hansen, H., Ho, E.W., Morishita, T.Y., Okuda, T., Sawa, T.R., Nakamura, R.M., Pederson, N.C., 1989. Epidemiologic and clinical aspects of feline immunodeficiency virus infection in cats from the continental United States and Canada and possible mode of transmission. J. Am. Vet. Med. Assoc. 194, 213–220.