Investigating an etiologic role for *Felis catus* gammaherpesvirus 1 in high grade, large cell lymphoma

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Hypothesis

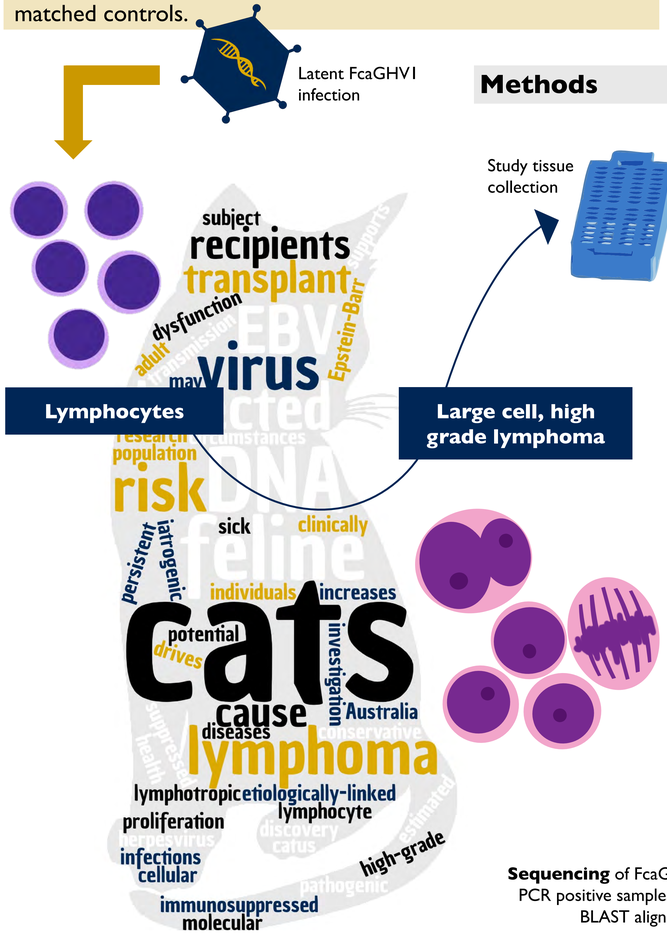
Felis catus gammaherpesvirus 1 (FcaGHV1) is causally-linked to post-transplant lymphoma or high grade, large cell lymphoma in domestic cats.

Introduction

The first gammaherpesvirus of domestic cats, **FcaGHV1**, was discovered in 2014.[1]

FcaGHV1 is a **worldwide** endemic lymphotropic infection detected in the blood of 10-19% of cats.[1-5] Gammaherpesviruses in humans and other animals cause persistent infections that are typically clinically latent but, in circumstances such as immune dysfunction or following cross-species transmission, gammaherpesviruses can cause **lymphoma** and other **fatal diseases**.[6] Lymphoma is the most common neoplasm in cats. Several prevalence studies have established that immunosuppressed cats, such as those either co-infected with feline immunodeficiency virus (FIV), feline leukemia virus (FeLV), or organ transplant recipients iatrogenically immunosuppressed, are more at risk for developing lymphoma. Emergence of any neoplasia in the context of immunosuppression is consistent with viral-associated cancer, which in humans is ~15% of the global cancer burden. The prevalence of **viral oncogenesis** in veterinary species is unknown. Whether FcaGHV1 plays a role in feline post-transplant lymphoma (PTL) or other high grade, large cell lymphomas is the subject of this investigation.

AIM: To determine whether FcaGHV1 DNA is detected more frequently in high grade, large cell lymphomas than in tissue matched controls.



Methods

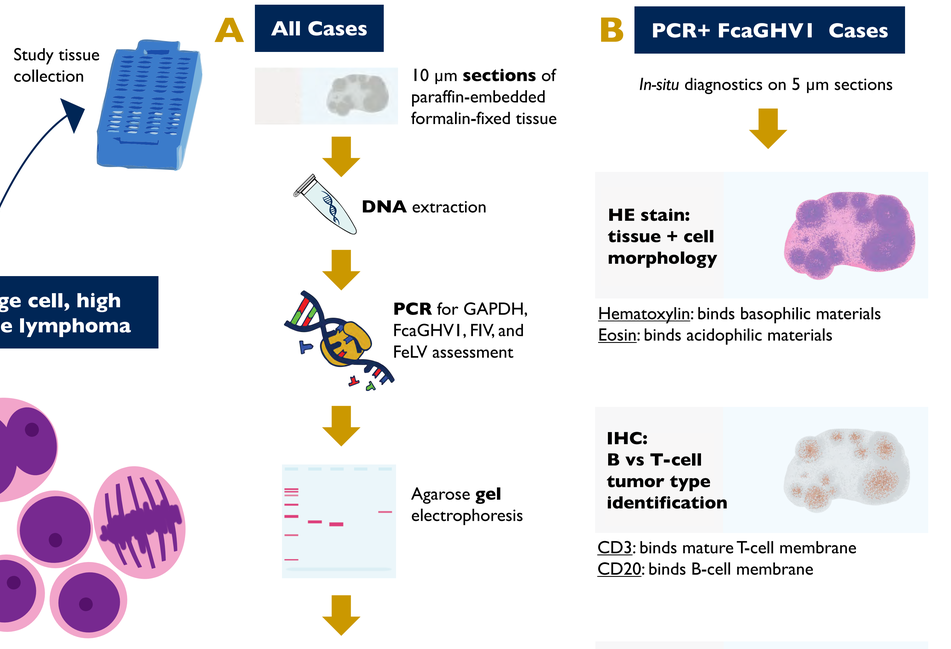
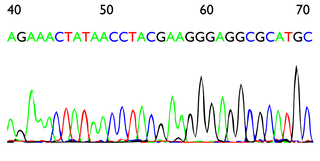


Figure 3. Example of a high quality chromatogram



Sequencing of FcaGHV1 PCR positive samples and BLAST alignment

Figure 1. Representative PCR results by agarose gel electrophoresis: case groups GIT (#1-20), PTL (#21-27), and HLL (#35) with a positive control (+), and negative controls (-H: hood, -B: bench, and -LN: FcaGHV1 negative cat lymph node). A) GAPDH and FcaGHV1: B) FIV and FeLV. Positives indicated by **golden arrows** and false positives by the **black arrow**.

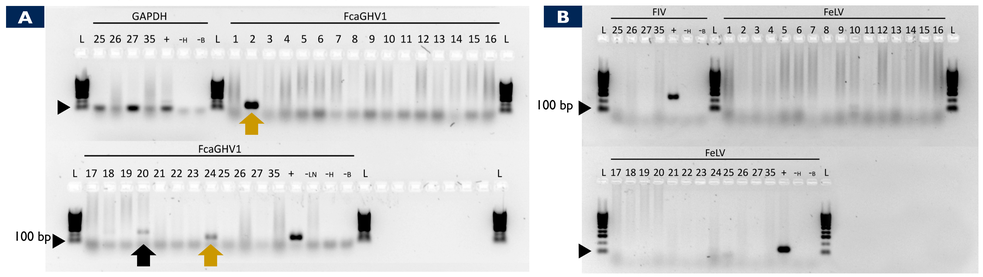


Table 1. PCR detection of viral DNA in feline lymphoma cases

Group	Cases	FcaGHV1	FIV	FeLV
PTL[a]	7	1	0	0
GIT[b]	20	1	0	0
HLL[c]	35	0	0	---
NSL[d]	20	1	---	---
LBL[e]	9	0	3	1
Total	91	3	3	1

[a]post-transplant lymphoma, [b]gastrointestinal large cell lymphoma, [c]T-cell rich, B-cell Hodgkin's like lymphoma, [d]nasal lymphoma, and [e]large B-cell lymphoma

Table 2. GAPDH, FcaGHV1, FIV, and FeLV specific primers for PCR assessment

Primer Set	Oligo	Sequences (5'-3')	Length (bp)	Reference
Feline GAPDH[a]	GAPfwd	AAGGCTGAGAACGGGAAAC	80	Beatty et al, 2014
	GAPrev	CATTTGATGTTGGCGGGATC		
FcaGHV1[b]	GH-3 F	unpublished data available upon request		
	GH-3 R			
FIV[c]	P-15-1	GTGATATACCAGAGACTTTA	271	Hohdatsu et al, 1992
	P-15-2	TTTACTGTTTGAATAGGATA		
FeLV[d]	FeLV_U3-exo_f	AACAGCAGAAGTTTCAAGGCC	80	Tandon et al, 2005
	FeLV_U3-exo-r	TTATAGCAGAAAGCGCGCG		

[a]feline glyceraldehyde-3-phosphate dehydrogenase, [b]*Felis catus* gammaherpesvirus 1, [c]feline immunodeficiency virus, [d]feline leukaemia virus

Conclusion

The discovery of novel oncogenic viruses raises the possibility for improved prevention and treatment of domestic feline cancers. With an estimated 200 million currently infected cats,[4,11] elucidating the potential epidemiology and pathology of FcaGHV1 is critical for supporting the health of our companion felines.

This study has isolated FcaGHV1 DNA in gastrointestinal lymphoma tissues for future investigations and provide support for a causal link between high grade, large cell lymphoma and FcaGHV1 infection in domestic cats. Future studies will be directed by our results to increase or decrease suspicion that FcaGHV1 is a candidate oncogenic gammaherpesvirus.

Results

91 domestic cats from American veterinary hospitals with high grade, large cell lymphoma were assessed for the detectable presence of FcaGHV1 DNA in paraffin wax-imbedded formalin fixed tissues by polymerase chain reaction (PCR), immunohistochemistry (IHC), and *in-situ* hybridization (ISH).

PCR positive cases (Figure 1A) were sequenced and identified using BLAST (#2, 20, and 24) except case #69 (NSL) (Table 1). Case #2 (GIT) and 24 (PTL) had >98% identity with FcaGHV1.[15] Case #20 (GIT) shared 86-88% identity and was treated as a false positive. All tissues were negative for FIV or FeLV DNA by PCR.

Cases #2 (small intestine section) and #24 (lymph node and small intestine section) were analyzed by HE stain, IHC, and ISH. Case #2 was positive by ISH for FcaGHV1 ORF 50, with no hybridization of the scrambled (unrelated) probes with similar nucleotide content. Probe localization was within the nuclei of infiltrative sheets of non-epitheliotropic round cells spanning from the mucosa to mural tissues. Positive cells were large (~10-14 µm) with expanded nuclei and distinct nucleoli located within the T-cell lymphoma population. Additionally, a few randomly dispersed macrophages within the tumor population contained probe within their nuclei and cytoplasm.

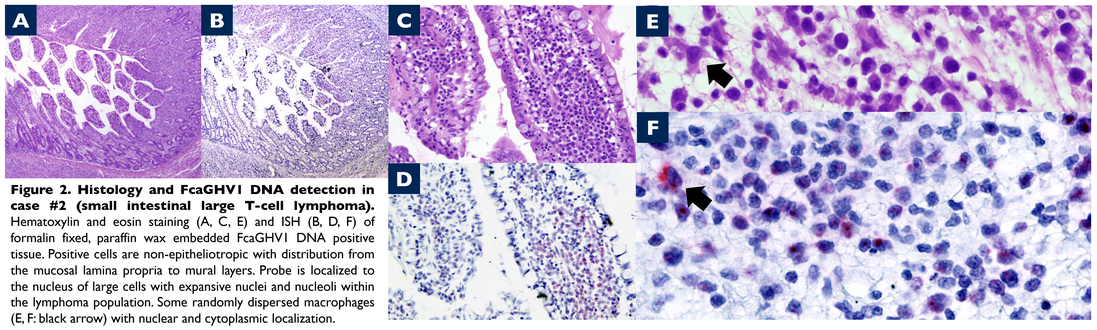


Figure 2. Histology and FcaGHV1 DNA detection in case #2 (small intestinal large T-cell lymphoma). Hematoxylin and eosin staining (A, C, E) and ISH (B, D, F) of formalin fixed, paraffin wax embedded FcaGHV1 DNA positive tissue. Positive cells are non-epitheliotropic with distribution from the mucosal lamina propria to mural layers. Probe is localized to the nucleus of large cells with expansive nuclei and nucleoli within the lymphoma population. Some randomly dispersed macrophages (E, F: black arrow) with nuclear and cytoplasmic localization.

Discussion

The epidemiology and pathology of FcaGHV1 is continuing to be discovered, but the known **risk factors** for infection include: adult, male, sick, reproductively intact, haemoplasma positive, and FIV or FeLV positive status.[1,2,5] The population for this study included cats with **high grade, large cell lymphoma**, providing the"sick" status. All other risk factors were unknown for most of the population. Due to the limited population signalment and only three FcaGHV1 positive cases in different groups and time points, this is a **descriptive study** for guiding future work. We will focus on discussing the possible epidemiology and pathology of FcaGHV1 based on our findings.

The current study was the first assessment of potential **tissue specificities for FcaGHV1**, specifically high grade, large cell lymphomas, since FcaGHV1 was originally characterized and studied in blood samples.[1,2,5] Single PTL and GIT cases were positive by PCR for FcaGHV1 DNA, but only the **large, T-cell GIT case contained positive cells by ISH**. Positive cells were large with expanded nuclei and visible nucleoli, and were **part of the lymphoma population**, supporting a descriptive correlation of FcaGHV1 with high grade, large cell lymphoma. Only one case out of 7 total high grade, large, T-cell GIT lymphomas were FcaGHV1 positive. Assessing for FcaGHV1 DNA presence by PCR could produce false positives, and the other 6 GIT cases will be assessed by ISH in future work.

Low FcaGHV1 detection is also supported by the case selection criteria decreasing the infection risk factors for the study groups. Cats in the PTL group had assumed high quality medical care and health screening,[9] including seronegative FIV and FeLV status that decreased risk of FcaGHV1 infection.[2,5] Donor cats are additionally in optimal health and are likely screened for FIV or FeLV, decreasing the risk of being FcaGHV1 positive and subsequent transmittance the virus to recipients. The case selection criteria for GIT, HLL and NSL groups is unknown, with unknown or limited signalment. Overall, the preliminary screens for PTL group and the unknown signalment for the other groups **decreased the risk of infection** with FcaGHV1, explaining the low detection in the sampled population.

Based on our findings, **FcaGHV1 may be localized to high grade, large, gastrointestinal T-cell lymphoma** in domestic cats.

References

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