Feline Infectious Keratitis Leading to Eye Rupture – Molecular Determination of Possible Pathogens

Diana Delgadillo, BS, and Becky Morrow, DVM

Duquesne University, Department of Biological Sciences and Frankie's Friends Cat Rescue

BACKGROUND

Upper respiratory infections (URIs) are common in cats. Typical signs of URI include sneezing, nasal discharge, ocular discharge, and conjunctivitis, inflammation of the sclera and lining of the eyelids. Keratitis, inflammation of cornea, is a less common sign of a URI, and can cause scarring and rupture of the eye. Feline herpesvirus 1 (FHV-1) is currently considered to be the most common primary pathogen associated with the ocular manifestations of URI, however, *Chlamydophila felis* (*C. felis)* and *Mycoplasma felis* (*M. felis*) are also prevalent in cats with conjunctivitis. Feline calicivirus (FCV) and other opportunistic bacteria may also be present.

PURPOSE

Figure 1: Severe, bilateral conjunctivitis and keratitis with mucopurulent discharge.

The purpose of this study was to identify the viral and bacterial pathogens present in eyes that had ruptured due to suspected infectious disease (ie. non-traumatic). Based on the results of previous studies, it was hypothesized that the most prevalent pathogens present in the ruptured eye tissue samples would be *M. felis* and FHV-1, with possible co-infection by FCV and *C. Felis.*

MATERIALS AND METHODS

Collection: Tissues used in this study were collected and stored at –20°C following surgical enucleation. Positive controls for FHV-1 and FCV, and *C. felis* were obtained from Nobiac: Feline 1-HCPCh vaccine containing modified live virus and *C. felis* (Merck).

Extraction: DNA and RNA were extracted from tissue samples and positive controls using EasyPrep™ DNA/RNA Miniprep Kit (Bioland Scientific), RNeasy® Mini Kit (Qiagen), and the method previously described by Boom et al. (1990).

Quantitation: Quantification and assessment of purity was performed with the NanoDrop Lite Spectrophotometer (Thermo Scientific), followed by normalization to a concentration of 15ng/µL.

PCR: A master mix was created for PCR using 5µL of 1X PCR buffer, 1µL of dNTPs, 1µL of a forward universal bacterial primer ITSF, 1µL of reverse universal bacterial primer ITSReub, 3mM $MgCl_2$, 1µL of TAQ polymerase, and 15µL of Nanopure water for each sample. 20 µL of extracted DNA was then added to a reaction tube with 30µL of master mix and run on the Applied Biosystems GeneAmp® PCR System 9700 using the following parameters: 1 hold at 94°C for 3 minutes, 35 cycles of denaturing at 94°C for 45 seconds, annealing at 55°C for 1 minute, and extension at 72°C for 2 minutes, with a final extension at 72°C for 7 minutes. A second master mix was created using the same components with designed FHV-1 forward and reverse primers in place of the universal bacterial primers. PCR parameters were the same, with the exception of a 50°C annealing temperature. Products were analyzed on a 1% agarose gel using ethidium bromide.

MATERIALS AND METHODS CONTINUED

qPCR and qRT-PCR: qRT-PCR for FCV was performed using Genesig® Feline Calicivirus Kit (Primerdesign™ Ltd) following manufacturer's instructions, with analysis on the MyGo Mini qPCR instrument (Arura Genomics™). A second master mix for FHV-1 qPCR was created using the AzuraQuant™ Green Fast qPCR Mix LoRox protocol with the same designed FHV-1 primers. 2µL of each DNA sample was added to 0.1 mL labeled tubes for analysis. The parameters on the instrument used were an enzyme activation at 95°C for 2 minutes, 45 cycles of denaturation at 95°C for 10 seconds with an anneal/extension at 60°C for 30 seconds, and a melting point analysis at 60°C.

Next Generation Sequencing: Next Generation Sequencing using the Illumina MiSeq platform was performed following PCR amplification of sample DNA using universal bacterial primers with barcoded forward primer, agarose gel electrophoresis, gel extraction with QiaQuick PCR Purification Kit (Qiagen), quantification, and normalization and pooling of samples.

Bioinformatics: Sequences were analyzed through the Linux-based HHMI computer cluster at Juniata College using QIIME. Sequences were quality filtered and assigned OTUs. Taxa were identified to the genus level via the UCLUST algorithm and Greengenes v. 13.8 database.

RESULTS

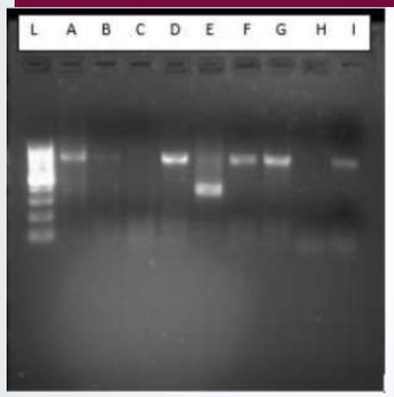


Figure 2: Results of PCR amplification using universal bacterial primers on tissue samples showing seven samples with an approximately 800 bp band.

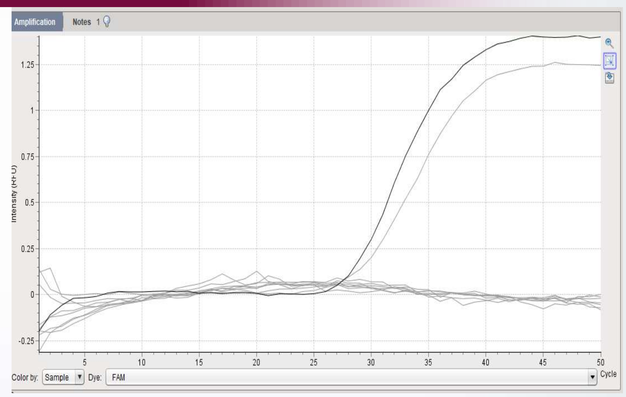
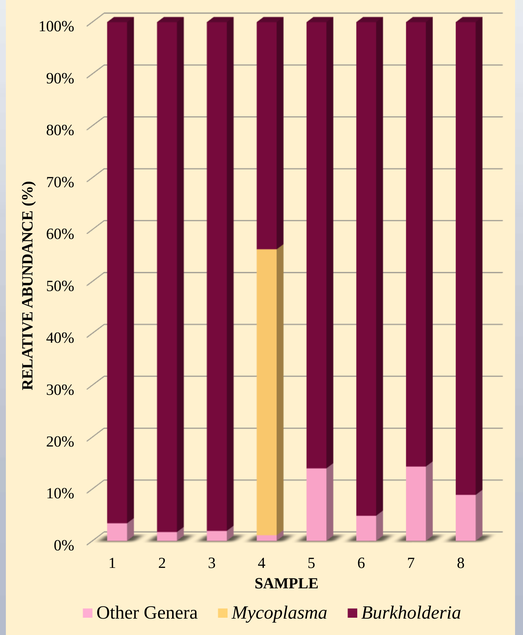


Figure 3: qPCR graph showing RFU vs Cycle number indicating a positive amplification result of Sample 7 for FCV.

Table 1: FHV-1, FCV, *M. felis*, *C. felis*, and *Burkholderia* Detection and Relative Abundance

Sample Number	FHV-1	FCV	*M. felis* Relative Abundance	*C. felis* Relative Abundance	*Burkholderia* Relative Abundance
1	N	N	0	0	97%
2	N	N	< 1%	0	98%
3	Y	N	< 0.01%	0	98%
4	Y	N	55%	0	44%
5	Y	N	<1%	0	86%
6	Y	N	< 0.1%	0	95%
7	N	Y	< 0.01%	0	86%
8	N	N	0	0	91%

Graph 1: Relative Abundance vs. Sample Showing Bacterial Genera Found in Each Sample



CONCLUSIONS

Because FHV-1, FCV, *C. felis* and *M. felis* are the most common pathogens associated with feline eye infections and URI, it was expected that one or more of these pathogens would be found in the ruptured eye tissue samples.

None of these pathogens, however, were found in all of the tissue samples, with only one positive for FCV, one for *M. felis* (at a level above 1% relative abundance), four for FHV-1, and none for *C. felis*. This suggests that co-infection with FHV-1, FCV, *C. felis*, *M. felis*, or another bacterial species is not necessary for development of infectious keratitis and eye rupture.

Bacteria from the genus *Burkholderia* was present in high relative abundance in all eight samples. The high abundance of *Burkholderia*, without a co-pathogen in each sample, highly suggests that *Burkholderia* is the primary cause of the infectious keratitis and eye rupture.

Very few cases of cats with *Burkholderia* infections have been reported. To the authors' knowledge, this is the first report of *Burkholderia* associated conjunctivitis and keratitis leading to eye rupture in cats. Further research will focus on determination of the species, phenotype, and virulence factors of the bacteria and prevalence of *Burkholderia* in cats with conjunctivitis and keratitis.

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