Accurate PCR diagnosis of feline infectious peritonitis

Rationale: Feline coronavirus (FCoV) infection is ubiquitous in domestic cat populations. As yet unknown mutations result in extra-intestinal replication of the virus, causing feline infectious peritonitis (FIP), a highly fatal immune-mediated disease. The uncertainty about the genetic polymorphisms that convert FCoV to FIP virus (FIPV) severely impedes ante-mortem FIP diagnosis by PCR. We have established a highly sensitive and specific real-time PCR assay for detection of subgenomic mRNA of the FCoV M gene (FIP matrix gene mRNA PCR), which identifies replicating FCoV in extra-intestinal specimens, the functional principle that characterizes FIPV. While FIP diagnosis by this PCR is very frequently requested from the Auburn University Molecular Diagnostics Laboratory, the overall diagnostic sensitivity and specificity of this assay needs to be ascertained. Recently, a conversion concept of FCoV to FIPV was proposed by which mutations within the S1/S2 motif of the FCoV spike protein reduce the ability of the cellular proteinase, furin, to cleave the spike protein. This mutation would alter the FCoV cell tropism towards monocyte/ macrophage infectivity, and thus convert FCoV to FIPV. We developed a S1/S2 motif real-time PCR assay (FIP S1/S2 gene PCR) to test this hypothesis by sequencing of these PCR products.

Hypothesis/Objectives: We hypothesize that the assumptions described above are correct, and will test them in the proposed investigation. Our objectives are to find the most accurate ante-mortem diagnosis of FIP by 1) clinically validating the FIP M gene mRNA PCR; 2) determining the associative strength of the S1/S2 motif mutation with FIPV by S1/S2 gene PCR and sequencing; and 3) establishing FIP diagnosis with highest predictive value as an optimal combination of relevant clinical symptoms, hematological/serum biochemical parameters, and FIPV PCRs.

Study Design: 1) Conduct a case-control study to evaluate the specificity and sensitivity of the FIP M gene mRNA PCR using ≥ 200 each FIP-positive and -negative specimens obtained from submitting veterinarians. 2) Evaluate the correlation of mutations in the S1/S2 motif with FIP in a) ≥ 100 each clinically confirmed positive FIP cases obtained for objective 1; and b) by comparing S1/S2 mutation frequency between populations of ≥ 100 FIP-biased S1/S2 amino acid sequences (positive specimens in the FIP M gene mRNA PCR) and ≥ 100 FIP-unbiased sequences (fecal swabs from juvenile cats obtained from the Humane Society in Montgomery, AL). 3) Correlate cases of objectives 1 and 2 with clinical/hematological/serum biochemical parameters, and determine by principal component and cluster analyses the combination with FIPV PCRs for highest FIP diagnostic predictive value.

Preliminary Data: We tested FIP M gene mRNA PCR-positive FIP-biased and -unbiased FCoV populations with the FIP S1/S2 gene PCR and sequenced the amplification products of the two populations. Among the total of 46 peritoneal fluid specimens from the FIP-biased population, 20 showed a canonical S1/S2 amino acid motif (RS/ARRS), whereas 26 (57%) showed a mutant motif with at least one amino acid change. Conversely, out of 26 fecal swabs from the FIP-unbiased population, only a single S1/S2 motif (4%) showed an amino acid change. The mutation frequency between the populations is highly significantly different ($p < 0.0001$; two-tailed Fisher exact test). This finding suggests that mutant S1/S2 motifs play a role in FCoV to FIPV conversion. Nevertheless, in the FIP-biased population, there are still 20 out of 46 specimens (43.5%) with an unchanged canonical amino acid sequence of the S1/S2 motif. This strongly suggests that decreased furin cleavage efficiency is not required and not the sole determinant of converting FCoV to FIPV.

Expected Results: Evaluation of sensitivity and specificity of the FIP M gene mRNA PCR can be achieved by correlation with the clinical FIP outcome. FIP-biased populations will show increased S1/S2 motif mutation, indicating that abrogated furin cleavage associates with FCoV to FIPV conversion, but FIPV diagnoses without S1/S2 mutation will suggest additional conversion mechanisms. Principal component and cluster analyses will reveal a set of critical clinical/hematological/serum biochemistry parameters in FIP, and combined with the FIPV real-time PCR assays, deliver the most accurate ante-mortem diagnosis of FIP.

Ethical Assurances: All animal procedures have been approved by the Auburn University Institutional Animal Care and Use Committee (IACUC).

Potential Impact for Animal Health: The combination of the FIPV real-time PCR assays and critical clinical/hematological/serum biochemistry parameters of FIP should at long last provide definite ante-mortem diagnosis for FIP. This will limit suffering of FIP cats due to timely euthanasia after correct diagnosis, will save countless cats from euthanasia due to false-positive diagnoses, and will accelerate research for FIP therapeutics.