Laboratory Diagnosis of Coronavirus Infections in Ferrets

Roger K. Maes, Annabel G. Wise, Michael M. Garner, Matti Kiupel
Coronavirus Characteristics

- Glycoproteins of envelope:
  - S – Spike
  - M – Membrane
  - E – Envelope

- Genome: + sense RNA
  - non-segmented
  - single-stranded
  - 27-31 kb

- RNA enclosed in capsid of N protein monomers
Coronavirus Phylogeny

Epizootic Catarrhal Enteritis (ECE)

- New diarrheal disease of ferrets (1993)
- First described on the East Coast of the U.S.
- “Green slime disease”, “Green diarrhea”, or “The Greenies”
- Clinical signs: lethargy, anorexia, vomiting, followed by profuse diarrhea
- Mortality rate 5%, Morbidity rate 100%
- Disease more severe in older ferrets
- Present throughout the U.S. and several countries
Epizootic Catarrhal Enteritis (ECE)
Ferret Enteric Coronavirus (FRECV)

- Novel group 1 coronavirus associated with epizootic catarrhal enteritis (ECE) in ferrets (Wise et al., 2006 Virology 349: 164-174)
Ferret Systemic Coronavirus (FRSCV)

Clinicopathologic Features of a Systemic Coronavirus-Associated Disease Resembling Feline Infectious Peritonitis in the Domestic Ferret (Mustela putorius)


- FIP-like disease observed in ferrets in Europe and the U.S. (2002-2007)
- Common clinical findings: anorexia, weight loss, lethargy, CNS signs, palpable intra-abdominal mass or masses
- FIP-like lesions: systemic pyogranulomatous inflammation in several abdominal and thoracic organs
FIP-like Disease in Ferrets

Widespread nodular foci on serosal surfaces of organs.

Widespread nodular foci on serosal surfaces of organs.
Ferret Systemic Coronavirus (FRSCV)

Comparative sequence analysis of the distal one-third of the genomes of a systemic and an enteric ferret coronavirus (Wise et al. Virus Research 149 (2010) 42-50)
Laboratory Diagnostic Methods

- Immunohistochemistry (IHC)
- In situ hybridization (ISH)
- RT-PCR
FECV Detection by IHC
FECV Detection by ISH
FRSCV Detection by IHC
Consensus RT-PCR Assays for Coronaviruses

- Highly degenerate primers targeting:
  - **Product size**
  - **Polymerase** 251 bp
  - **Spike** 628 bp
  - **M-N region** 735 bp

Coronavirus Genome ~ 30 kb
Gel-Based RT-PCR Assay to Detect FRECV or FRSCV

- Oral swab specimens
- Fecal swab specimens

-113 bp
SYBR Green-Based Real-Time RT-PCR Assay for FRECV or FRSCV Detection

- Quantitect SYBR-Green RT-PCR Kit (QIAGEN, Valencia, CA)
- iCycler iQ System with detection software v. 3.1 (Bio-Rad Laboratories, Hercules, CA)
Comparative sequence analysis of the distal one-third of the genomes of a systemic and an enteric ferret coronavirus

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## Sequence Comparison of the 3’ Genomic ends of FSCV MSU-1 and FECV MSU-2

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S-Gene Genotype-Specific Diagnostic RT-PCR on Clinical Samples

FRSCV-specific primers

FRECV-specific primers
rRT-PCR Assays to Differentiate between FRSCV and FRECV
Moses, stop the crap