Sequencing-Based Identification of a Novel Coronavirus in Ferrets with Epizootic Catarrhal Enteritis and Development of Molecular Diagnostic Tests

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Coronaviruses

- Genus under Coronaviridae family
- Irregularly-shaped particles
- 60-220 nm in diameter
- Club-shaped peplomers
  - Embedded in the envelope
  - “Crown/corona-like” appearance
Coronaviruses

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

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

- RNA enclosed in capsid of N protein monomers
Coronavirus Antigenic Groupings

I (mammalian)  Human coronavirus 229 E  
TGEV of swine  
Feline infectious peritonitis virus  
Canine coronavirus  

II (mammalian)  Human coronavirus OC43  
Mouse hepatitis virus  
Bovine coronavirus  
Porcine hemagglutinating encephalomyelitis virus  

III (avian)  Infectious bronchitis virus of chickens  
Bluecomb disease virus of turkeys
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
Etiology of ECE

• Earlier investigations implicated a coronavirus (Williams, et. al., 2000 in JAVMA vol.217 no.4)
  • based upon immunohistochemistry (using feline coronavirus mab) and transmission electron microscopy; virus isolation was unsuccessful.
  • Lack of “nucleotide sequence-based evidence” to substantiate findings
Sequencing-based Determination of the Causative Agent of ECE

- 3 consensus RT-PCR assays for coronaviruses
- Samples: Total RNA extracted from diarrheic feces of ferrets affected with ECE
- Cloning and sequencing of the PCR products
- BLAST and sequence analyses
Consensus RT-PCR Assays for Coronaviruses

Highly degenerate primers targeting:

- Polymerase: 251 bp
- Spike: 628 bp
- M-N region: 735 bp

Coronavirus Genome ~ 30 kb
Products of Expected Sizes Amplified

**Pol** 251-bp

**Spike** 628-bp

**M-N** 735-bp

TGEV
Results of BLAST Analysis

Amplicon sequence data confirmed to be “authentic coronavirus” sequences

<table>
<thead>
<tr>
<th>Ferret sequences:</th>
<th>% Similarity to TGEV</th>
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<tbody>
<tr>
<td>Pol (208 bp)</td>
<td>76.4%</td>
</tr>
<tr>
<td>Spike (586 bp)</td>
<td>60.8%</td>
</tr>
<tr>
<td>Membrane (336 bp)</td>
<td>66.7%</td>
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Complete Capsid Gene Sequencing

• Entire capsid (N) gene sequence derived by 3’ RACE (1,125 bp-ORF)

• Nucleotide sequence similarities:
  • 51.6% (CCV)
  • 48.7% (TGEV)
  • 48.2% (FCoV)
  • 34.5% (HCoV 229E)
This novel coronavirus has been named Ferret Enteric Coronavirus (FECV).
Development of Molecular Diagnostic Tests for ECE

• FECV-specific RT-PCR assay
• Target: portion of capsid gene
• Product size: 113 bp
• Formats: Gel-based Real-time (SYBR Green)
• For rapid detection of the virus in fecal or oral swab specimens
Gel-Based RT-PCR Assay to Detect FECV

Oral swab specimens

Fecal swab specimens

-113 bp
SYBR Green-Based Real-Time RT-PCR Assay for FECV

- Quantitect SYBR-Green RT-PCR Kit (QIAGEN, Valencia, CA)
- iCycler iQ System with detection software v. 3.1 (Bio-Rad Laboratories, Hercules, CA)
Development of Molecular Diagnostic Tests for ECE

- In-situ hybridization (ISH) assay
- Digoxigenin-labelled FECV nucleocapsid gene-specific probe
- Detection system: anti-digoxigenin ab conjugated with alkaline-phosphatase and substrate NBT/X-Phos
Development of a Serologic Test for ECE: Recombinant Baculovirus Technology

- **FECV capsid gene cloned in baculovirus (BAC-to-BAC Baculovirus Expression System by Invitrogen, Carlsbad, CA)**

- **Expression of FECV capsid protein in insect cells (SF9) detected by IPMA (immunoperoxidase monolayer assay)**
Detection of FECV Capsid Protein Expression in SF9 cells

Feline anti-FCoV

Ferret anti-FECV

Bovine anti-BVDV
Conclusions

- This is the first definitive identification of a coronavirus in ferrets, based upon RT-PCR, sequencing and sequence analyses.
- The causative agent of ECE is a novel coronavirus, designated as Ferret Enteric Coronavirus (FECV).
- Several molecular diagnostic tests to diagnose FECV infection in ferrets have been developed.
Molecular characterization of a novel coronavirus associated with epizootic catarrhal enteritis (ECE) in ferrets

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Abstract

A novel coronavirus, designated as ferret enteric coronavirus (FECV) epizootic catarrhal enteritis (ECE). Initially, partial sequences of the polym using coronavirus consensus PCR assays. Subsequently, the complete sequ 3’ terminus of the FECV genome were obtained. Phylogenetic analysis, spike, and membrane proteins, and full sequence of the nucleocapsid pro coronaviruses. FECV is more similar to feline coronavirus, porcine trans epidemic diarrhea virus and human coronavirus 229E. Molecular data | coronaviruses associated with clinical cases of ECE.
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