Viral Causes of Enteric Disease in Ferrets: A Comparison Between Ferret Enteric Coronavirus and Ferret Rotavirus Type C Infections in Ferrets with Diarrhea

RC Smedley, AG Wise, M Kiupel, RK Maes
Diarrhea in Ferrets

- Sudden diet change
- Gastric ulcers: due to stress or *Helicobacter*
- Foreign body ingestion
- Neoplasia: Lymphosarcoma
- Eosinophilic enteritis
- IBD
- Viruses: Coronavirus, Rotavirus
- Bacteria
- Parasites
Ferret Enteric Coronavirus (FECV)

- Epizootic catarrhal enteritis (ECE)
- High morbidity $\rightarrow$ 100%
- Low mortality (< 5%)
- Most severe in older ferrets
  - Mean age ~ 4 years in one study
  - Juvenile ferrets – mild or subclinical disease
- Initial clinical signs (48-72 hrs post exposure):
  - Lethargy, inappetence, vomiting
Ferret Enteric Coronavirus (FECV)

- Enveloped, positive-strand RNA virus
- Virions are pleomorphic and range from 60-220 nm in diameter
- Detected in feces and saliva using an N gene-specific RT-PCR
- IHC – detect antigen
- ISH – detect nucleic acid
- IFA – detect antibody
Phylogenetic tree, based on deduced partial amino acid sequences of the polymerase proteins
Ferret Rotavirus

- **Typical Rotavirus: Group A**
- **Atypical Rotaviruses: Groups B-G**
- **Anecdotal reports of Group A rotavirus diarrhea in juvenile ferrets**
  - Recent case submission
  - Sequencing of VP6 gene most similar to a bovine group A rotavirus (97% nucleotide identity)
- **Atypical rotavirus isolated from neonatal ferrets with diarrhea (Torres-Medina 1987)**
- **Ferret Rota C-MSU**
Ferret Rotavirus C-MSU

- 9 ferrets euthanized & presented for necropsy
- ~1 week old
- Undetermined sex
- History of diarrhea
Additional Diagnostics

- Bacterial culture of small intestine: negative
- RT-PCR for group A rotavirus: negative
- RT-PCR assay specific for group C rotavirus: positive
  - Consensus primers designed from alignment of VP6 gene sequences of porcine, bovine and human strains
  - Target 182 bp-region of VP6 gene
  - Strain designated Ferret Rota C-MSU
VP6

• Major structural inner capsid protein on outer surface of single-shelled particles
• Encoded by genome segment 6 in group A rotaviruses and by gene 5 in group C rotaviruses
• Commonly targeted - highly immunogenic and antigenic
• Sequencing:
  • One ORF of 1,185 nucleotides $\rightarrow$ 395 a.a. protein (same as other group C rotaviruses)
  • Predicted m.w. of 44.5kDa (similar to other group C rotaviruses)
### Sequence Analysis

<table>
<thead>
<tr>
<th>Group</th>
<th>% Nucleotide</th>
<th>% Amino Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group C:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shintoku (bovine strain)</td>
<td>81.1</td>
<td>92.2</td>
</tr>
<tr>
<td>Cowden (porcine strain)</td>
<td>78.6</td>
<td>90.9</td>
</tr>
<tr>
<td>Jajeri (human strain)</td>
<td>76.9</td>
<td>88.1</td>
</tr>
<tr>
<td>Preston (human strain)</td>
<td>76.7</td>
<td>88.4</td>
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<tr>
<td><strong>Group A:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avian rotavirus A</td>
<td>41.5</td>
<td>41.5</td>
</tr>
<tr>
<td>Bovine rotavirus A</td>
<td>42.8</td>
<td>40.8</td>
</tr>
</tbody>
</table>
Amino Acid Substitutions (x100)

- Ferret Rota C-MSU
- Shintoku (bovine)
- Cowden (porcine)
- Jajeri (human)
- Preston (human)
- Avian Rota A
- Bovine Rota A
Ferret Rotavirus C-MSU

- Highly prevalent in facility
- Additional cases have been identified
- Transmission: contact with infected individuals or contaminated environment
- No evidence of cross species transmission from ferrets ↔ other species
- Reports in other species of rotavirus reassortants crossing species
Rotaviruses

• Cause of diarrhea worldwide
• Humans and many animal species
  • Calves, piglets, rats, mice, foals, lambs, kids, rabbits, monkeys, poultry, dogs, cats
• Children and young animals
• Group C rotavirus
  • Nursery piglets (Saif et al. 1980)
• Cattle, humans, dogs, ferrets
**FECV versus Ferret Rota C-MSU**

- **FECV**
  - High morbidity
  - Low mortality
  - Adults
  - Classic green mucoid diarrhea
  - Atrophic enteritis

- **Ferret Rota C-MSU**
  - High morbidity
  - Low mortality
  - Juveniles
  - Pale yellow liquid diarrhea
  - Atrophic enteritis
Diagnosis of Viral Enteritis

- Standard ELISA and immunofluorescence tests only identify group A rotaviruses
- Standard negative staining methods for TEM can’t differentiate groups or strains
- PCR and sequence analysis needed to confirm Rotavirus and identify group
Additional Diagnostics

- Sequencing and sequence analysis of VP6
- Automated DNA sequencing
- BLAST analysis
- Sequence assembly and analysis (Clustal W method)
- Phylogeny trees (Lasergene biocomputing software)
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 polyoma virus using coronavirus consensus PCR assays. Subsequently, the complete sequenced the 3’ terminus of the FECV genome were obtained. Phylogenetic analysis of spike, and membrane proteins, and full sequence of the nucleocapsid pro coronaviruses. FECV is more similar to feline coronavirus, porcine transmissible diarrhea virus and human coronavirus 229E. Molecular data for coronaviruses associated with clinical cases of ECE.

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