Development and Evaluation of Molecular Techniques for the Diagnosis of Epizootic Catarrhal Enteritis of Ferrets

A. Wise, Matti Kiupel, R. Maes
Clinical Signs

- profuse green mucous diarrhea
- dehydration, anorexia, weakness
- more severe disease in older ferrets than younger ferrets
- 100% morbidity and 5% mortality
Histopathology

- Atrophy, blunting, and fusion of villi
- Vacuolar degeneration and necrosis of enterocytes
Etiology of ECE

- First described in 1993 in US and Canada
- Retrospective study on 119 naturally infected ferrets (Williams et al., 1999)
- Coronavirus identified in feces and enterocytes by TEM
- IHC: reacted with antibody against feline coronavirus
- Suspected a novel Ferret Enteric Coronavirus
Study Goals

- Evaluate PCR and In situ hybridization as diagnostic tools for ECE on:
  - feces, saliva, blood (PCR)
  - tissues (ISH)
- Infect ferrets with FECV to:
  - Study viral shedding
  - Aquire stock of virus for future studies
  - Reproduce ECE
Hypothesis

- H1: RT-PCR can be used to detect FECV in feces of ferrets affected by ECE.
- H2: RT-PCR can be used to detect FECV in saliva of ferrets affected by ECE.
- H3: In-situ hybridization can be used to detect FECV in tissues of ferrets affected by ECE.
Methods

• Acquired 13 ferrets for inoculation study
• all ferrets were positive for FECV at day 0
• Rectal and oral swabs were collected from each ferret daily
• PCR was performed on fecal and oral samples from day 0 to day 26
• Necropsies were performed and tissue samples collected for In-situ hybridization
  • spleen, intestines, lymph nodes, and salivary glands.
RT-PCR on Feces

FECV was detected consistently in feces of affected ferrets from day 0 to day 26.
RT-PCR on Saliva

FECV was detected in saliva of affected ferrets from day 0 to day 26.

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<th>Ferret</th>
<th>10</th>
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Immunohistochemistry and In Situ Hybridization

IHC

ISH
Immunohistochemistry and In Situ Hybridization
Results

- RT-PCR was determined to be a specific and sensitive tool for FECV detection
- PCR product was sequenced to validate results
- FECV was detected by RT-PCR in feces and saliva, but not blood
- Strength of the FECV signal did decrease significantly over the 26 days
- FECV was detected by ISH in small intestine
Conclusions

- FECV specific PCR and ISH were developed
- both tests were valuable in detecting FECV
- FECV is shed in saliva as well as in the feces of affected ferrets
- During a natural infection viral shedding lasts for at least 26 days
Future Goals

• Obtain SPF-ferrets (free of FECV)
• Inoculate SPF-ferrets with FECV to reproduce ECE
• Study transmission of FECV from jills to offspring:
  • lactogenic immunity, environmental contamination, vertical and horizontal transmission
• develop an antibody test
Molecular characterization of a novel coronavirus associated with epizootic catarrhal enteritis (ECE) in ferrets

Annabel G. Wise a,b,*, Matti Kiupel a,b,c, Roger K. Maes a,b,d

a Diagnostic Center for Population and Animal Health, Bm. 136E, 4125 Beaumont Road, Lansing, MI 48909, USA
b Michigan State University, Ea
c Department of Pathobiology and Diagnostic Investigation, dDepartment of Microbiology and Molecular Genetics, Mi

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Abstract

A novel coronavirus, designated as ferret enteric coronavirus (FECV) epizootic catarrhal enteritis (ECE). Initially, partial sequences of the polyom using coronavirus consensus PCR assays. Subsequently, the complete sequ the 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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