

# Occurrence of ferret enteric coronavirus in Brazil (Preliminary Report)

## *Ocorrência de coronavírus entérico de ferrets no Brasil - Nota Prévia*

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### Abstract

Ferret enteric coronavirus (FECV) is associated to the epizootic catarrhal enteritis (ECE) in ferrets (*Mustela putorius furo*). In this study, we report the occurrence of this agent in four diarrheic stool samples of domestic ferrets, analyzed by negative staining transmission electron microscopy and a specific RT-PCR assay targeting the nucleocapsid (N) gene. These findings are the first report of FECV in Brazil and address the importance of this virus on the etiology of enteric disorders in ferrets.

**Keywords:** Ferret enteric coronavirus. Epizootic catarrhal enteritis. Ferret. *Mustela putorius furo*. Coronavirus.

### Resumo

Coronavírus entérico de furões (FECV) é associado à enterite catarral epizootica (ECE) em furões (*Mustela putorius furo*). Neste estudo, relatamos a ocorrência deste agente em quatro amostras fecais diarreicas de furões domésticos, analisadas por microscopia eletrônica de transmissão (contrastação negativa) e RT-PCR específica e direcionada ao gene de nucleocapsídeo (N). Estes achados constituem o primeiro relato de FECV no Brasil e remetem para a importância deste vírus na etiologia de quadros entéricos nestes animais.

**Palavras-chave:** Coronavirus entérico de ferrets. Enterite catarral epizootica. Furão. *Mustela putorius furo*. Coronavirus.

Coronaviruses are classified in the order *Nidovirales*, family *Coronaviridae*, which comprises the genera *Coronavirus* and *Torovirus*<sup>1</sup>. The genus *Coronavirus* is subdivided in three groups (I, II, and III) according to epitopes of envelope glycoproteins, nucleotide sequences, and natural hosts<sup>2</sup>. The virions are enveloped, mainly spherical or pleomorphic, with an average diameter of 75-160 nm, with club-shaped surface projections around 20 nm long<sup>3</sup>, and the genome is a non-segmented positive-sense single-stranded RNA of approximately 32kb that forms a helicoidal nucleocapsid in association with the nucleoprotein (N)<sup>4</sup>.

In ferrets, a coronavirus was associated to epizootic catarrhal enteritis (ECE)<sup>5</sup>, mostly causing enteric

disorders. Wise, Kiupel and Maes<sup>6</sup> reported that the virus associated with ECE is a novel group I coronavirus, denominated as ferret enteric coronavirus (FECV), as well designed a pair of FECV-specific RT-PCR primers targeting a 113-bp region of N (nucleocapsid) gene. This study describes the detection of FECV present in diarrhea episodes in domestic fer-

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rets (*Mustela putorius furo*), a fact not yet reported in Brazil. Stool samples were collected from four diarrheic pet ferrets from São Paulo state, Southeastern Brazil, in 2005. These animals were from different owners, all of them with intermittent diarrhea, with ages varying from four months to six years. The four stool samples were submitted to negative staining electron microscopy according to Brenner e Horne<sup>7</sup>. Briefly, stool samples were prepared as 50% suspensions in PBS (PBS 0.1M pH 7.0). A total of 40  $\mu$ L of the supernatant was added to copper grids with carbon stabilized supporting film of 0.5% collodium in amyl acetate for 10 min at room temperature and negative stained with 2% ammonium molybdate (pH

5.0). The samples were observed with Philips EM208 transmission electron microscope.

For RT-PCR, stool samples were prepared as 50% (w/v) suspensions in PBS (PBS 0.01M/BSA 0.1% pH 7.2) and clarified at 12,000xg/30 min at 4°C, and the supernatant was stored at -20 °C until analysis. Reverse transcription (cDNA synthesis) was carried out at 42 °C for 60 minutes in a reaction mix with 1xFirst Strand Buffer (Invitrogen™), 1 mM of each dNTP, 10 mM DTT, 1  $\mu$ M of each FECV-specific primer targeted to *N* gene (as described by Wise, Kiupel and Maes<sup>6</sup>), 7  $\mu$ L of RNA extracted with TRIzol (Invitrogen™) (according to the manufacturer's instructions and denatured at 95 °C for 5 minutes) and 200 U of M-

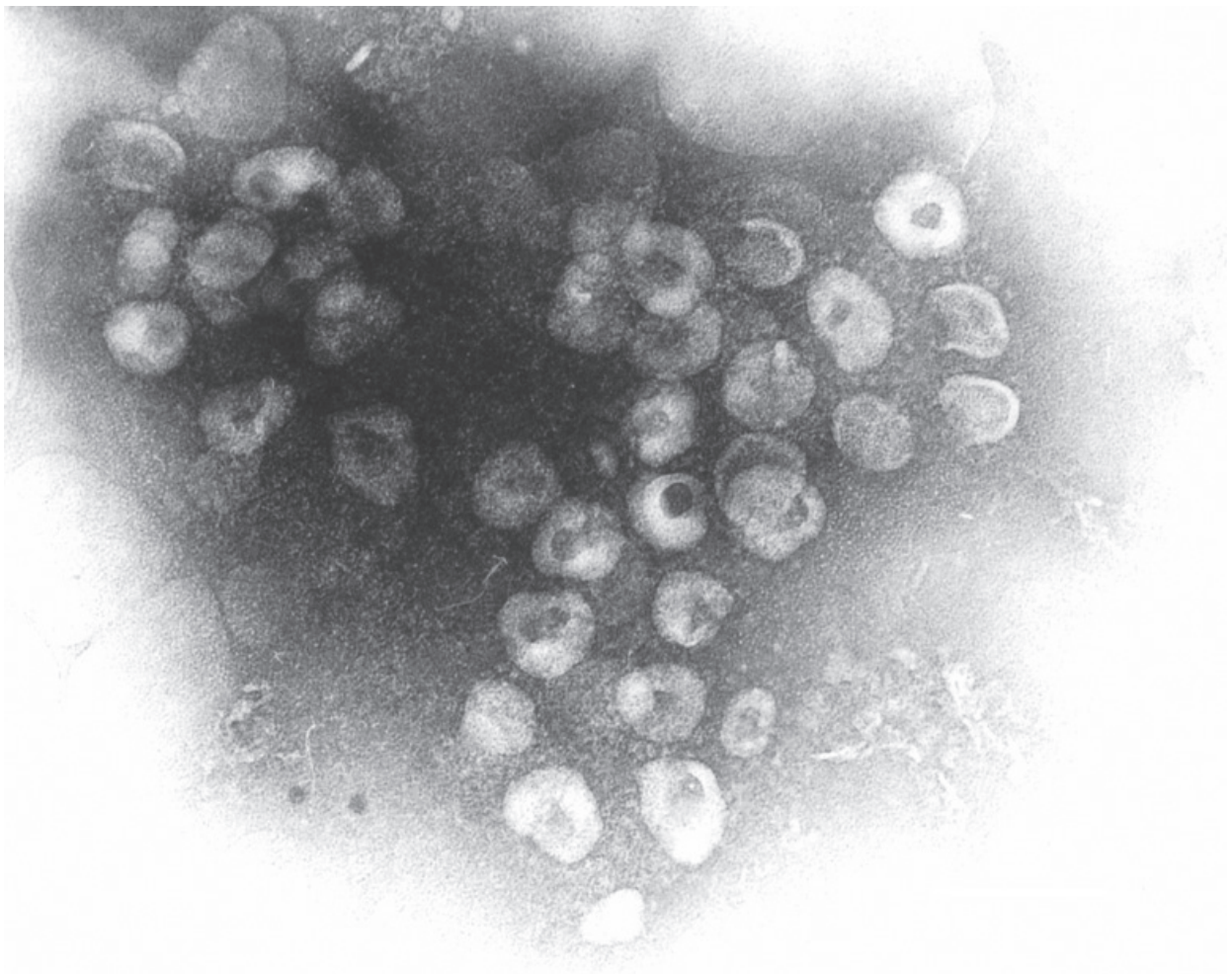


Figure 1 - Negative staining electron microscopy of diarrheic stool sample of a domestic ferret, showing coronavirus-like particles. Scale bar indicates 180 nm

MLV Reverse Transcriptase (Invitrogen™) in a 20 µL final reaction volume. Next, 5 µL of cDNA was added to the PCR mix with 1xPCR Buffer (Invitrogen™), 0.2 mM of each dNTP, 0.5 µM of each FECV-specific primer<sup>6</sup>, 1.5 mM MgCl<sub>2</sub>, 25.25 µL of ultra-pure water, and 1.25 U Taq DNA polymerase (Invitrogen™) in a 50 µL final reaction volume and submitted to 40 cycles of 94 °C for 30 s, 53 °C for 30 s and 72 °C for 30 s, followed by 72 °C for seven minutes for final extension.

Ultra-pure RNase-free water was used as negative control; Bovine Coronavirus (BCoV) Kakegawa Strain and Canine Coronavirus (CCoV) RNA templates, both previously positive to a coronavirus consensus PCR<sup>8</sup>, were also tested. Furthermore, in order to avoid any laboratory contamination, each step (RNA extraction, PCR, and electrophoresis) was carried out in a separate room with separate materials. The PCR products were resolved on a 2% agarose gel stained with 0.5 µg/mL ethidium bromide and those resulting in the 113-bp predicted fragment were considered positive.

The electron microscopy revealed pleomorphic virus particles, measuring approximately 120 nm in diameter (range from 90 nm to 140 nm) and distinctive

spike projections close to 20 nm in length, compatible with a coronavirus-like morphology (Figure 1). All fecal samples, but not BCoV and CCoV, generated FECV-specific 113-bp fragments by RT-PCR, assuring the specificity of the assay and excluding the possibility of infections by these two agents.

These results demonstrate in a conclusive way the presence of ferret coronavirus amongst the sampled animals, and nucleotide sequencing data of the strains reported herein became necessary to elucidate new emerging aspects of the virus. For instance, it was described a coronavirus-associated systemic disease in ferrets with similar lesions as seen on the feline infectious peritonitis (FIP), mostly granulomatous<sup>9,10</sup>. According to Garner et al.<sup>10</sup>, the virus present on the samples were closely related, but not identical to the previously characterized ferret coronavirus strain (FECV-MSU1)<sup>6</sup>, hypothesizing a recent mutation or shift in the FECV.

As a conclusion, this is the first report of FECV in Brazil and address the importance of this virus on the etiology of enteric disorders in ferrets as already reported in other countries.

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