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Address for correspondence: Tara C. Smith, 200 Hawkins Drive C21F GH, Iowa City, IA 52242, USA; email: tara-smith@uiowa.edu

# Sequence Analysis of Feline Coronaviruses and the Circulating Virulent/Avirulent Theory

**To the Editor:** Feline coronaviruses (FCoVs) occur as 2 pathotypes, feline infectious peritonitis virus (FIPV) and feline enteric coronavirus (FECV). FECV is common in cats, causing mild transient enteritis in kittens, but is asymptomatic in adult cats. In contrast, FIPV occurs sporadically but is lethal. It replicates in monocytes and macrophages and rapidly disseminates throughout the body causing systemic immunopathologic disease (*1–4*).

The relationship between FECV and FIPV has become a matter of debate. Genetic and animal experimental evidence indicates that FIPV arises by mutation from FECV in the intestinal tract of a persistently infected cat; the virus thereby acquires the monocyte or macrophage tropism that enables it to spread systemically and cause FIP (*5–7,8*). According to another view, the 2 pathotypes circulate independently in the field. This circulating virulent/avirulent FCoV theory recently was advocated by Brown et al. (*9*). Their conclusion was based on sequence analyses of parts of the viral genome including the matrix (M) gene, phylogenetic analysis of which revealed reciprocal monophyly of the sequences obtained from FIP cases versus those of asymptomatic FECV-infected animals. In addition, the authors suggested 5 aa residues in the M protein to represent potential diagnostic markers for distinguishing virulent FIPV from avirulent FECV (*9*).

To try to verify the findings of Brown et al. (*9*), we determined and analyzed M genes from 43 FCoV genomes, 20 of which came from cats in single-cat households, and 23 from cattery animals. The latter group consisted of 10 asymptomatic healthy cats (FECV; test specimens: feces) and 13 dead cats with FIP confirmed through pathology (FIPV; test specimens: organs, ascites). These animals came from 8 catteries. FECV and FIPV cases were found in 7 (designated A to G); the remaining cattery (H) provided 2 cats with FIP. The genomes from individually living cats were from 15 FIPV- and 5 FECV-infected animals.

Using specific primers (sense 5′-CGTCTCAATCAAGGCATATAATC CCGACGAAG-3′, antisense 5′-CAG TTGACGCGTTGTCCCTGTG-3′), we amplified the same 575-bp M gene fragment as studied by Brown et al. (*9*). GenBank accession numbers for the FCoV M gene sequences determined in this study are HQ738691–HQ738733. When compared by phylogenetic analysis, the nucleotide sequences of FIPV and FECV M genes distributed into paraphyletic patterns rather than in monophyletic clusters (Figure, panel A).

Thus, as we observed earlier for the 3c gene (*10*), M gene sequences generally clustered according to the cattery from where they originated, irrespective of their pathotype (e.g., FECV 586 and FIPVs 584 and 585 from cattery A; FECV 620 and FIPVs 615 and 622 from cattery G; FECV 10 and FIPV 8 from cattery F). Such a distribution pattern is consistent with the mutation theory, according to which FIPVs originate from FECVs and are thus closely related (*7,9*). Exceptions in this picture were FIPV 9 in cattery F and FECVs 406 and

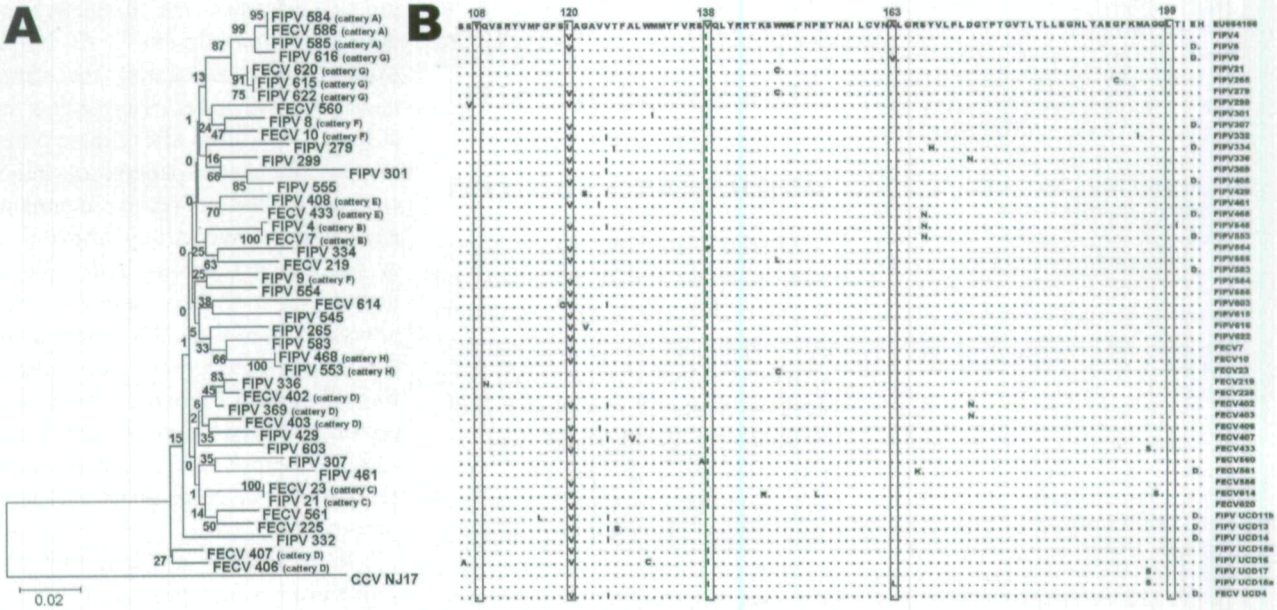


Figure. A) Phylogenetic relationships of feline coronaviruses (FCoVs) detected in feces of healthy cats and in organs/ascites of cats with feline infectious peritonitis. Alignment of the matrix (M) gene sequences was used to generate a rooted neighbor-joining tree with the M gene sequence of canine coronavirus strain NJ17 (Genbank accession no. AY704917) as outgroup. Bootstrap confidence values (percentages of 1,000 replicates) are indicated at the relevant branching points. Branch lengths are drawn to scale; scale bar indicates 0.02 nucleotide substitutions per site. Viruses detected in cattery animals are indicated by a cattery designation after the virus identification number. B) Alignment of amino acid sequences of partial M proteins of the FCoVs from panel A, as compared with a feline infectious peritonitis virus (FIPV) reference sequence (top line) published by Brown et al. (*9*) (GenBank accession no. EU664166), and with 8 American FCoV sequences (bottom) published by Pedersen et al. (*8*). The 5 aa residues at positions 108, 120, 138, 163, and 199, suggested by Brown et al. (*9*) as potential diagnostic sites, are boxed.

407 in cattery D, presumably caused by multiple FCoV lineages in these open catteries (an open cattery is one in which cats can move in and out, usually for breeding purposes).

We also examined the 5 aa sites in the M protein identified by Brown et al. (*9*) as being potentially diagnostic of FIP. An alignment of the relevant part of the polypeptide sequence, comprising the presumed signature residues at positions 108, 120, 138, 163 and 199, is shown in the Figure, panel B, for all FIPV and FECV genomes sequenced in this study. Within this sample collection, we observed complete sequence conservation at positions 108 and 199, virtually complete conservation (1 difference) at position 163. The 2 aa identities (Val and Ile) found at position 120 and 138 occurred with similar frequencies in FIPV and FECV (position 120: Ile in 16/36 [44%] FIPVs and in 6/14 [43%] FECVs; position 138: Ile in 29/36 [81%] FIPVs and in 12/14 [86%] FECVs). These observations do not indicate the slightest tendency of sequence segregation among the 2 pathotypes. In the alignment of the Figure, panel B, we also included M protein sequences translated from several FCoV genomes from the Americas, 7 FIPV, and 1 FECV (*8*). The comparison does not reveal peculiarities indicative of geographic segregation. Hence, our data do not confirm the diagnostic potential of the M protein sequence nor do they support the suggested role of the membrane protein in FIP pathogenesis (*9*).

Informative as it may be, comparative sequence analysis will eventually not be sufficient to answer the FECV/FIPV question. What will be needed is a reverse genetics system to generate and manipulate the FCoV genome as well as a cell culture system to propagate the viruses, both of which have thus far not been achieved.

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**Hui-Wen Chang, Herman F. Egberink, and Peter J.M. Rottier**

Author affiliation: Utrecht University, Utrecht, the Netherlands

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Address for correspondence: Peter J.M. Rottier, Virology Division, Department of Infectious Diseases and Immunology, Veterinary Faculty, Utrecht University, Yalelaan 1, 3584 CL Utrecht, the Netherlands; email: p.rottier@uu.nl



# Effects of Vaccination against Pandemic (H1N1) 2009 among Japanese Children

**To the Editor**: We report findings from a household-based study on the protective effects of vaccination against pandemic (H1N1) 2009 among Japanese children. In Japan, prioritized vaccination started in October 2009, focusing on health care workers, pregnant women, persons with underlying diseases, and children 1–9 years of age. Only nonadjuvant split vaccines (inactivated) produced by 4 manufacturers (Denka Seiken, Tokyo, Japan; Kaketsuken, Kumamoto-shi, Japan; Kitasato, Tokyo, Japan; and Biken, Suita-shi, Japan) were used by the end of January 2010 (*1*). Because the protective effects of vaccination at the individual level are best measured by household data (*2*), we conducted a retrospective household survey involving 1,614 nonrandomly sampled households (i.e., based on area sampling of households across Japan, according to the regional population size, with a total of 6,356 household members), in which the earliest cases were diagnosed from October 2009 to mid-February 2010. Our study aimed to assess vaccine-induced reductions in susceptibility and infectiousness among children by using the household secondary attack rate.

Influenza cases were defined as confirmed cases (i.e., diagnosed by real-time PCR) or influenza-like illness (ILI) cases (i.e., in febrile patients [≥37.5°C] with cough and/or sore throat). The cases had to meet the following inclusion criteria for analyses: 1) index case-patient and exposed persons in households were healthy children 1–9 years of age (households with <2 children were excluded), because age-specific susceptibility and infectiousness can greatly influence the frequency of household transmission (*3–6*); b) all exposed persons shared the same household with index case-patients for at least 1 of 7 days after illness onset of the index case-patient; c) index case-patient did not receive treatment with antiviral agents (e.g., zanamivir or oseltamivir) within 2 days after illness onset; d) time interval from illness onset of the index case-patient to that of subsequent case-patients was ≤7 days (*7,8*); and e) vaccinated persons received their first vaccination >28 days before illness onset (if index case-patient) or exposure (if not index case- patient).

In total, 251 children met the above criteria, comprising 109 index case-patients and 133 unvaccinated and 9 vaccinated exposed persons. The mean age was 6.4 ± 2.1 SD years. Among the 251 children, 15 (6.0%) had been vaccinated, and 169 (67.3%) had received a diagnosis of influenza. Confirmed cases accounted for 17.8% (30/169) of cases; 21 patients were the index case-patients in individual households. The mean age of patients with confirmed diagnoses was 6.5 ± 2.0 SD years and did not differ significantly from the ILI patients.

Let $SAR_{ij}$ represent the household secondary attack rate (SAR) with vaccination statuses of the index patient $j$ and exposed persons $i$ (where $i$ or $j$ is 0 or 1 for unvaccinated or vaccinated, respectively), and let $b$ represent both groups. Among 133 exposed unvaccinated children, ILI developed in 59, yielding an $SAR_{0b}$ of 44.4%. Among 9 exposed vaccinated children, ILI developed in 1 child, yielding an $SAR_{1b}$ of 11.1%. The difference between these SARs was marginally significant ($p = 0.08$ by Fisher exact test), and the susceptibility reduction was $1 - SAR_{1b}/SAR_{0b}$ = 75.0% (95% confidence interval [CI] –60.5% to 96.1%). Considering only exposures caused by unvaccinated first patients, $SAR_{00}$ and $SAR_{10}$ were 44.7% (59/132) and 0% (0/4), respectively.