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ネコカリシウイルス（FCV）の分子多様性に関する研究

Study on the molecule diversity of Feline Calicivirus (FCV)

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Abstract. A multicat household experienced an epidemic of feline calicivirus (FCV) infection. We analyzed molecular evolution of isolated FCVs by a phylogenic tree reconstructed around the genotype of FCV that was isolated from the cat of infectious origin. FCV was isolated from eight of 34 cats: two 1-year-old, male littermates that became latent carriers (Ao198-1 and Ao199-1); two 3-month-old littermates (Ao222-1 and Ao224-1); three 4-month-old littermates (Ao210-1, Ao212-1 and Ao213-1) and a 5-month old (Ao210-1). All the isolates belonged to the genogroup II, and their nucleotide sequences showed > 94% homology. They were subdivided into six distinct clusters by phylogenetic analysis, and Ao198-1, the source of infection, was most closely related to Ao199-1, then Ao212-1, Ao210-1, Ao214-1, Ao213-1, Ao222-1and Ao224-1 in this order. The similar result was obtained by phylogenic analysis of amino acid sequences. Sequence alignments of the isolates showed that the nonsynonymous substitution/total number of nucleotides ratio was < 40% in the regions A, B, D and F, and > 60% in the regions C and E. The nucleotide substitution/total number of nucleotides ratio was 33%, 12% and 15.6% at the region C and two hypervariable regions (5'and 3'HVRs) of the region E, respectively, and less than 10% at the other regions. For amino acids, the ratio was 50%, 24%, 10.3% and 25% at 5' and 3'HVRs of the regions C and E, respectively, especially high at the 5'HVR of the region C. The other regions were less than 10%. Our result suggested that the virus, while its transmission to the newborn cats, underwent frequent mutation especially at the regions C and E, suggesting these regions were most often involved in evolution of the FCV genome.

1. 目　的

Feline Calicivirus（FCV）感染症は猫の上部気道疾患であり，FCVは活動型の無症状感染の状態で，回復猫で持続感染しており，ウイルスが排泄されることがある。多頭飼育された猫のFCV感染症集団発生は，必ずしも母親から感染するとは限らず，他の個体から感染する可能性があるので，感染ルートを識別することが必要となる。ウイルスの配列から分子系統樹を作成，検討することにより，家族からの感染か否かを推定する方法が用いられているので，我々は，集団内感染が疑われる猫からの分離FCV株の塩基配列を決定し，分子系統樹を作成し，遺伝子解析により，家族内感染，母子間感染の頻度やリスクファクターを検討した。

2. 方　法

34匹を飼育する家庭内で分離されたFCVから，

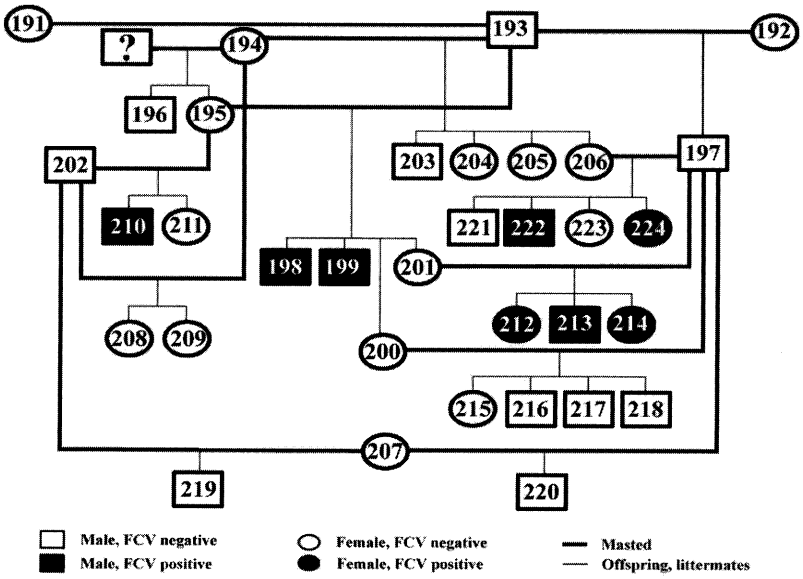

Fig. 1. Family tree of the 34 cats.

RT-PCRによりカプシド領域を増幅し，TAクローニングベクターにより，遺伝子クローニングを行い，塩基配列を決定した。NJ法により分子系統樹を作成し解析を行った。

3. 結果と考察

1）34匹中8匹（PERO, MIRO, BON, MARU, MOGU, KOKO, TEN, KUMI）から，ウイルスが分離され，それぞれAo198-1, Ao199-1, Ao210-1, Ao212-1, Ao213-1, Ao214-1, Ao222-1, Ao224-1とした（図1）。

2）FCVのカプシド領域を検出するプライマーとして，Sealのプライマーが用いられているが，Sealのプライマーで検出できなかったので，新たにプライマーを構築し（Aoのプライマー），検出を試みた所，目的の産物が検出された（図2）。

3）分離ウイルスのシークエンス解析により，ワクチン株（F9株）とのホモロジーが75.5％以下であること，それぞれのホモロジーが94％以上であることから，8匹に感染したFCVは，同一ウイルスであることが明らかとなった（図3）。

4）1年後のウイルス再分離では，同腹猫のPEROとMIROからウイルス分離がされており，この2匹が持続感染状態でウイルスを排泄していることが判明した。また，年齢がこの2匹のみ約1歳で，他の6匹は5ヶ月齢以下の幼猫でワクチンを接種していないもの（2匹）と接種しているもの（4匹）がおり，

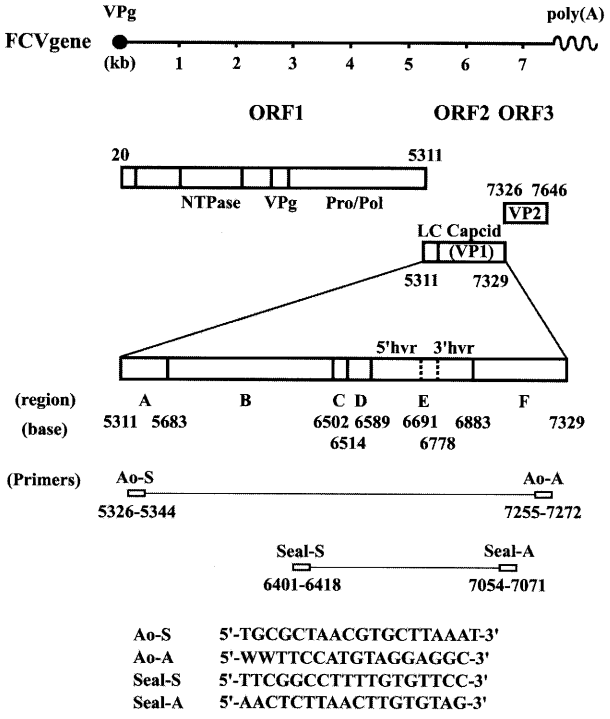

Fig.2. Genome map of FCV and PCR primers.

母親はいたが移行抗体の減少時期にあると思われ，それにより十分に防御できなかった可能性がある。これらのことを考慮に入れ，分子系統樹の樹形により，PERO（Ao198-1）が感染源となり，次いでMIRO（Ao199-1）→MARU（Ao212-1）→BON（Ao210-1）→KOKO（Ao214-1）→MOGU（Ao213-1）→TEN（Ao222-1），KUMI（Ao224-1）と伝播してい

AA \ DNA	Ao198-1	Ao199-1	Ao210-1	Ao212-1	Ao213-1	Ao214-1	Ao222-1	Ao224-1
Ao198-1	99.7-100 / 99.2-100	99.5-99.8	96.1-96.4	97.4-97.8	96.3-96.8	96.6-96.9	96.4-96.8	96.5-96.8
Ao199-1	99.1-99.8	99.8-100 / 99.7-100	96.3-96.5	97.6-97.9	96.5-96.9	96.8-97.0	96.6-96.9	96.7-96.9
Ao210-1	94.9-96.6	95.6-96.1	99.7-100 / 99.7-100	96.7-97.0	97.4-97.7	97.6-97.8	97.2-97.5	97.3-97.5
Ao212-1	96.2-97.0	96.8-97.3	95.9-96.2	99.6-99.8 / 99.5-99.7	96.9-97.4	97.1-97.4	96.7-97.1	96.8-97.1
Ao213-1	94.8-96.1	95.4-96.2	96.7-97.5	95.4-96.4	99.2-99.5 / 98.9-99.1	98.3-98.6	98.3-98.6	98.4-98.7
Ao214-1	95.4-96.1	96.1-96.2	97.6-97.9	95.9-96.1	97.3-97.8	99.4-99.6 / 99.4	98.3-98.5	98.4-98.6
Ao222-1	94.5-95.6	95.1-95.7	96.2-97.0	94.8-95.4	97.0-97.8	97.5-97.9	99.7-100 / 99.1-100	99.6-99.9
Ao224-1	94.4-95.6	95.4-95.7	96.5-97.0	95.1-95.4	97.3-97.8	97.8-97.9	99.1-100	99.7-99.9 / 99.5-100

Fig.3. Homology based on the nucleotide sequences and amino acid sequences of the isolated viruses.

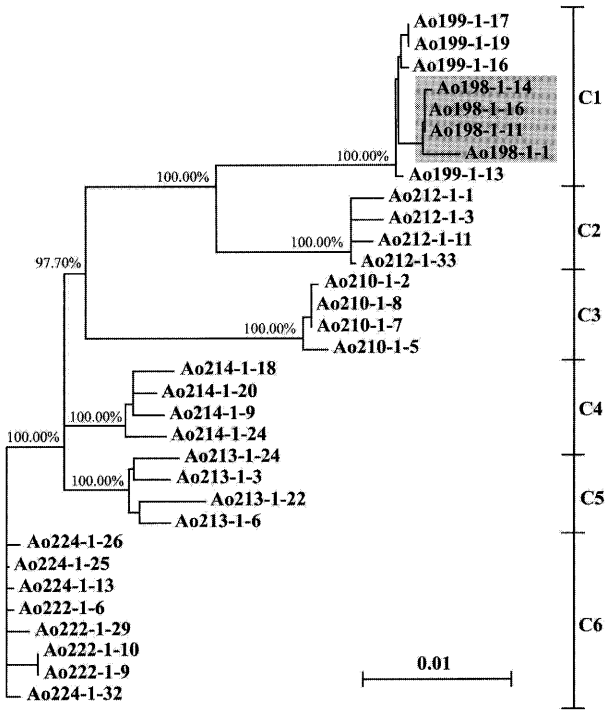

Fig. 4. Phylogenetic tree based on nucleotide sequences obtained by neighbor-joining (NJ) method for capsid fragments of the isolates. Branch lengths are proportional to the distances between the taxa. The values at the branch points indicate the percentage support for a particular node after 1000 bootstrap replicates were performed. Ao198 is the source of infections.

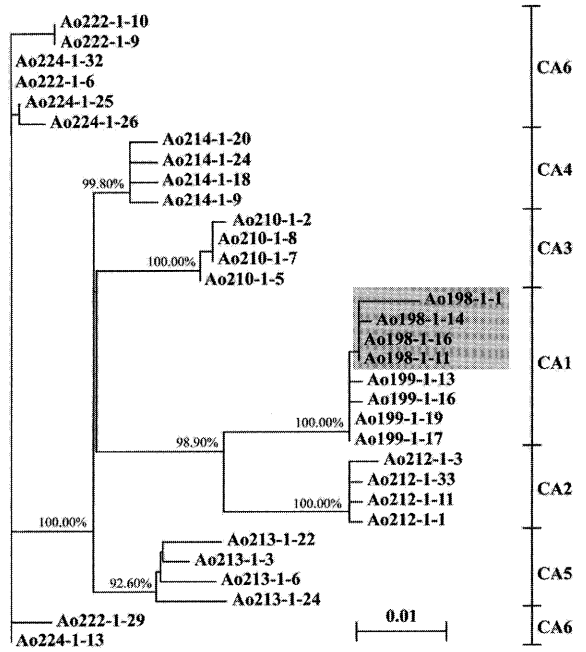

Fig. 5. Phylogenetic tree based on amino acid sequences obtained by neighbor-joining (NJ) method for capsid fragments of the isolates. Branch lengths are proportional to the distances between the taxa. The values at the branch points indicate the percentage support for a particular node after 1000 bootstrap replicates were performed. Ao198 is the source of infections.

ったと推察された（図4. 5）。

5）感染源とされるPEROやMIROはウイルスが分離された仔猫の親猫ではないが，一般的に雄は仔猫に興味を示すことはなく，仔猫の世話をするのは専ら雌猫であるので，母猫から仔猫への伝播が推測されたが今回は，雄成猫が感染源となり，仔猫へ感染するという結果となった。

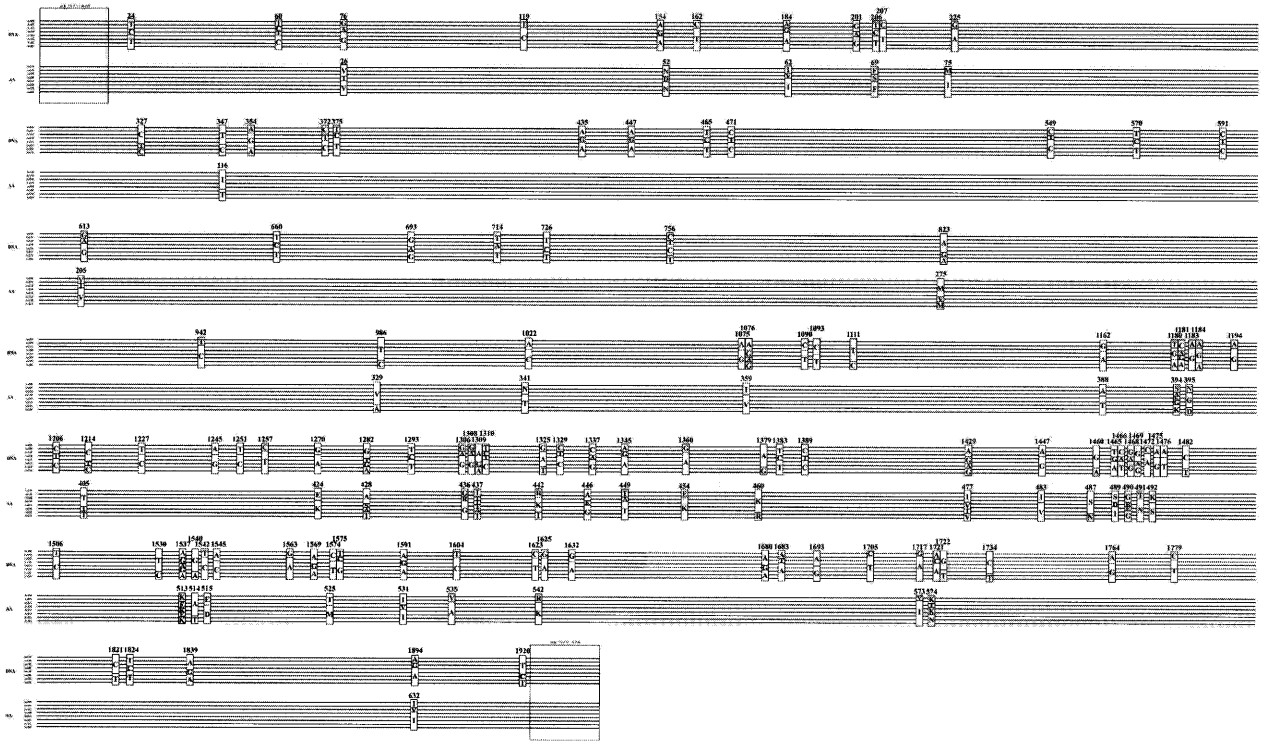

Fig.6. Alignment of the isolated viruses.

6）遺伝子解析では，中和抗原エピトープとされるC領域でヌクレオチドは33％，アミノ酸は50％，E領域でヌクレオチドは11.2％，アミノ酸は20％（5'hvr；11.8％, 23.5％, constant；5.7％, 10.3％, 3'hvr；15.6％, 25％, ヌクレオチド，アミノ酸の順）にそれぞれ変化していた。これら以外の領域は，ヌクレオチドが2.9～9.3％，アミノ酸が2.2～5.4％で低かった。これらは，抗原性と関与するC，E領域の変異が激しいという従来の報告と類似していた（図6）。

4. 要 約

一般的に雄は仔猫に興味を示すことはなく，仔猫の世話をするのは専ら雌猫であるが，今回は，雄成猫が感染源となり，仔猫へ感染するという結果となった。

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