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The Molecular Genetics of Feline Coronaviruses: Comparative Sequence Analysis of the ORF7a/7b Transcription Unit of Different Biotypes

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Feline coronaviruses (FCoVs) have been subdivided into feline enteric coronaviruses (FECVs) and feline infectious peritonitis viruses (FIPVs) on the basis of pathogenic properties. Serologically, a distinction has been made between type I and II FCoVs, the latter of which more closely resemble canine coronavirus (CCV). To gain more insight into the genetic relationships between different FCoV biotypes, we determined the nucleotide sequences of the ORF7a/7b transcription unit of nine strains. The following observations were made: (i) The sequences are 87–100% identical. In this part of the genome, type I and II FCoVs are more closely related to each other than to CCV. To explain the genetic and antigenic differences between the spike genes of type I and II FCoVs, we postulate that type II FCoVs have arisen by an RNA recombination event between a type I FCoV and CCV. (ii) The avirulent "FECV" strains UCD and 79-1683 are more similar to virulent "FIPV" strains than to each other. Our findings thus support the notion that FECV and FIPV are not different species but merely virulence variants. (iii) In contrast to FECV 79-1683, FECV UCD contains an intact ORF7b, indicating that ORF7b deletions are not a universal distinguishing property of FECVs. (iv) ORF7b deletions readily occur *in vitro*, correlating with loss of virulence. By reverse transcription–polymerase chain reaction analysis, we show that in naturally occurring FCoVs ORF7b is maintained. Thus, ORF7b seems to provide a distinct selective advantage during natural infection. © 1995 Academic Press, Inc.

INTRODUCTION

Feline coronaviruses (FCoVs) are enveloped, positive-stranded RNA viruses of wild and domestic felidae. They are associated with mild enteric infections but also with a fatal immune-mediated disease called feline infectious peritonitis (FIP; for a review see de Groot and Horzinek, 1995). FCoVs are closely related to coronaviruses of dogs (canine coronavirus, CCV) and swine (transmissible gastroenteritis virus, TGEV).

Up to 90% of the cats in catteries and 10–50% of the cats in single-cat households are FCoV-seropositive (Pedersen, 1976a; Loeffler *et al.*, 1978; Sparkes *et al.*, 1991, 1992; Addie and Jarrett, 1992a), but FIP occurs sporadically: only 5–10% of the seropositive cats actually develop the disease (Pedersen, 1976b; Addie and Jarrett, 1992a). It would thus appear that FIP is an infrequent manifestation of a common, inapparent infection. However, this view seems at odds with the fact that certain FCoV laboratory strains such as 79-1146 are highly virulent and in most cases cause FIP upon inoculation. Pedersen (1983) therefore proposed that there are actually two separate groups of FCoVs, i.e., virulent FIP-inducing viruses (FIPVs) that can cause systemic infections and avirulent feline enteric coronaviruses (FECVs) that remain restricted to the enteric tract. This hypothesis was supported by the isolation of two avirulent FCoV strains designated FECV UCD (Pedersen *et al.*, 1981b) and FECV 79-1683 (McKeirnan *et al.*, 1981).

Based on serologic studies yet another subdivision of FCoVs was made into types I and II (Pedersen *et al.*, 1984a; Hohdatsu *et al.*, 1991a, b). Type II FCoVs are antigenically more closely related to canine coronavirus than to the type I strains. Also in their *in vitro* growth characteristics, type II FCoVs are more similar to CCV (Pedersen *et al.*, 1984a). Type II infections account for about 5% and 20–30% of the FIP cases in the USA and Japan, respectively (Pedersen *et al.*, 1984a; Hohdatsu *et al.*, 1992).

Thus far, the type II FIPV strain 79-1146 is the best-characterized FCoV at the molecular level. Its genome is approximately 30 kb in length, the 3′-most 9 kb of which have been sequenced. The genes for the four structural proteins (S, E, M, and N) have been identified. Five additional ORFs have been found, two of which (ORF7a and 7b, formerly 6a and 6b; see de Groot and Horzinek, 1995) are located at the very 3′ end of the genome (Fig. 1; de Groot *et al.*, 1988). ORF7a codes for a small hydrophobic protein. In TGEV-infected cells, the 7a protein was found in the nucleus (Garwes *et al.*, 1989), but also in close association with the endoplasmic reticulum and the plasma membrane (Tung *et al.*, 1992). ORF7b, which is unique to FCoV and CCV (de Groot *et*

Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under Accession Nos. X90570–X90578.

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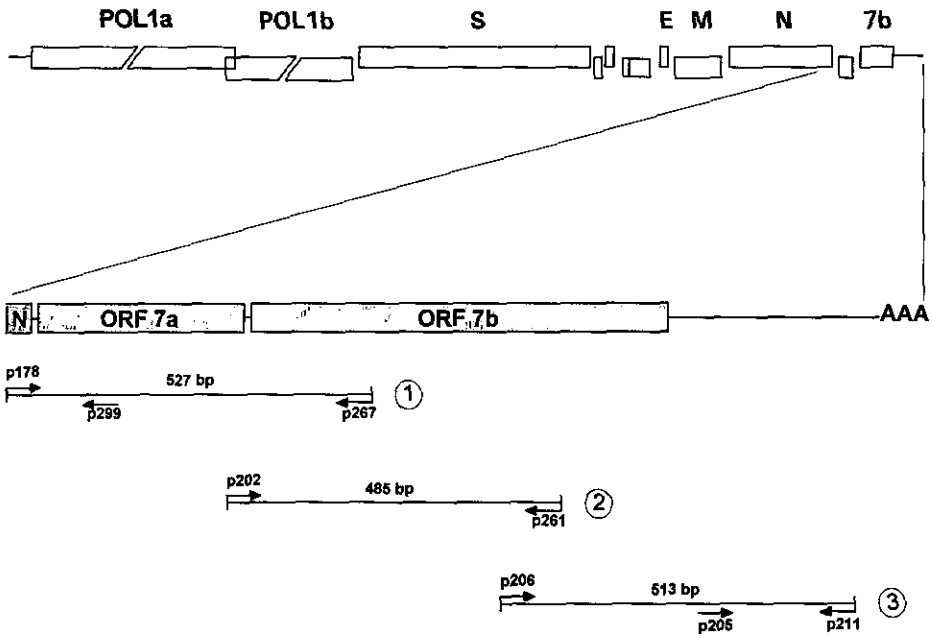

FIG. 1. Schematic outline of the strategy used for the sequence analysis of the 7a/7b transcription unit. The upper panel shows the organization of the FIPV strain 79-1146 genome. The genes for the polymerase polyprotein (POL1a and POL1b), for the structural proteins S, E, M, and N, and for the nonstructural protein 7b are indicated. The lower panel depicts the ORF7a/7b transcription unit and shows the positions of the oligonucleotides used for RT–PCR and sequencing. Arrows indicate the 5′ to 3′ orientation of the primers and the direction of the cycle sequence reactions. The circled numbers refer to the PCR products obtained when using primer sets 1 (p178 and p267), 2 (p202 and p261), and 3 (p206 and p211).

al., 1988; Vennema *et al.*, 1992b; Horsburgh *et al.*, 1992), encodes a nonstructural secretory glycoprotein of unknown function (Vennema *et al.*, 1992a).

The FECV strain 79-1683 is genetically very closely related to FIPV strain 79-1146, their spike genes being 95.5% identical (Reed *et al.*, 1993). The 7a/7b transcription unit is also well conserved, showing 93.3% nucleotide identity, but in strain 79-1683 the ORF7b gene is truncated by a 238-nucleotide deletion (Vennema *et al.*, 1992b).

Clearly, a better insight into the genetic relationships between the various FCoVs is crucial for understanding FCoV epidemiology and for the development of vaccines and diagnostic assays. For instance, it is possible to differentiate between FECV and FIPV? Do all FECVs have ORF7b deletions? How distinct are type I and type II FCoVs? To answer these questions, we have determined the nucleotide sequence of the ORF7a/7b transcription unit for nine additional FCoV strains. This collection included the type I FECV strain UCD and various type I and II FIPVs. We show that the different FCoV biotypes are indistinguishable in this part of the genome and discuss the implications for the evolution and classification of FCoVs. Furthermore, we provide evidence that ORF7b is readily lost during *in vitro* propagation but maintained during natural FCoV infection.

MATERIALS AND METHODS

Virus and clinical specimens

Details on the various FCoV laboratory strains used for reverse transcription–polymerase chain reaction (RT–PCR) and sequence analysis are listed in Table 1. Feces, ascitic fluid, or tissue samples were collected from 16 cats with a natural FCoV infection (Table 3).

RT–PCR and sequence analysis

RNA was extracted from tissue culture supernatant, feces, various tissue samples, and ascitic fluid and concentrated using a modification of the guanidinium isothiocyanate (GuSCN)–silica (SiO_2) protocol of Boom *et al.* (1990). RT–PCR was performed as described (Herrewegh *et al.*, 1995). Details of the oligonucleotide primers used for RT–PCR and sequence analysis are listed in Table 2. For sequence analysis, the amplified DNA products were purified by electrophoresis in 1% SeaPlaque GTG low-melting-point agarose gels (FMC BioProducts, Rockland, ME) run in 0.4× TAE (16 m*M* Tris–acetate, 0.4 m*M* EDTA). Cycle sequencing was performed directly on molten gel slices containing approximately 40 ng of the PCR product, using a cycle sequencing kit (Gibco BRL Life Technologies, Gaithersburg, MD).

Computer analysis of nucleic acid and amino acid sequences

The cDNA sequences were aligned pairwise using the PC-DOS HIBIO DNASIS software (Lipman and Pearson, 1985) from Pharmacia Biochemicals (Milwaukee, WI). Multiple alignments of nucleic acid and amino acid sequences were performed using the Pileup program (University of Wisconsin), which scores the similarity between every possible pair using a method similar to the one described by

TABLE 1

FCoV Strains

Strain	Virulence[a]	Type[b]	Specimen[c]	Reference	Source
FIPV					
79-1146	HV	II	Fcwf cells, TC	McKeirnan *et al.* (1981)	
TN406-LP[d]	HV	I	Fcwf cells, TC	Black, 1980	N. Pedersen
TN406-HP[d]	A	I	Fcwf cells, TC	Pedersen and Black (1983)	N. Pedersen
Dahlberg			Mouse brain	Osterhaus *et al.* (1978)	A. Osterhaus
UCD1/NW-1	HV	I	Fcwf cells, TC	Pedersen *et al.* (1981a)	N. Pedersen
UCD2	A	I	Fcwf cells, TC	Pedersen and Floyd (1985)	W. Corpai
UCD3	V	I[e]	Culture medium	Pedersen and Floyd (1985)	N. Pedersen
UCD4	V	II[e]	Culture medium	Pedersen and Floyd (1985)	N. Pedersen
UCD5		I[f]	Feline omentum	Pedersen (personal communication)	N. Pedersen
UCD6		II[f]	Feline omentum	Pedersen (personal communication)	N. Pedersen
Wellcome	HV		FEL cells, TC	O'Reilly *et al.* (1979)	D. Harbour
NOR15	HV	II	Fcwf cells, TC	Evermann *et al.* (1981)	J. Evermann
NOR15/tsDF2	A	II	Primucell FIP	Christianson *et al.* (1989)	SmithKline
FECV					
UCD	A	I	Feline feces	Pedersen *et al.* (1981b)	N. Pedersen
79-1683	A	II		McKeirnan *et al.* (1981)	
CCV					
Insavc-1				Horsburgh *et al.* (1992)	
K378				Vennema *et al.* (1992b)	
TGEV					
Purdue				Kapke and Brian (1986)	

[a] Biologic properties: HV, highly virulent; V, virulent; A, avirulent.
[b] Assignment according to Pedersen *et al.* (1984a).
[c] TC, tissue culture supernatant.
[d] LP, low-passage virus stock. HP, high-passage virus stock.
[e] Tentatively assigned to serotype I (Hohdatsu *et al.*, 1991a,b).
[f] Assignment according to Vennema *et al.* (1995).

Higgins and Sharp (1989). The similarity scores obtained for the nucleic acid sequences were used to create a dendrogram by the unweighed pair–group method using arithmetic averages according to Sneath and Sokal (1973). Gaps in the alignments were given the weight of a single nucleotide residue difference regardless of their length.

RESULTS

Sequence analysis of the 3′-most transcription unit

To study the genetic variation among FCoVs, we determined the nucleotide sequence of the ORF7a/7b transcription unit of nine FCoV laboratory strains. The pathogenic and serologic properties of these strains are listed in Table 1. Figure 1 shows a schematic representation of the FCoV genome and an outline of the sequencing strategy. By RT–PCR on virion RNA, three overlapping cDNA products were generated. The nucleotide sequences of the RT–PCR products were determined in both orientations by thermocycling sequence analysis.

The ORF7a/7b transcription unit was intact in eight of the strains. In FIPV strain UCD2, however, two deletions were observed in ORF7b covering positions 96–190 and 245–339. The nucleotide sequences were compared to each other and to those of FIPV 79-1146 (de Groot *et al.*, 1988), FECV 79-1683 (Vennema *et al.*, 1992b), the CCV strains K378 (Vennema *et al.*, 1992b), and Insavc-1 (Horsburgh *et al.*, 1992). From the data summarized in Figs. 2A and 2B the FCoVs emerge as a rather homogeneous group, sharing 87–100% sequence identity. In this part of the genome they are more closely related to each other than to the CCV strains studied thus far. The CCV-like type II FCoV strains 79-1146 and 79-1683 (Pedersen *et al.*, 1984a; Hohdatsu *et al.*, 1991a, b) cannot be distinguished from type I FCoVs. There may be geographic differences, however: Wellcome, a British strain (O'Reilly *et al.*, 1979), is more distant from the other, mainly American FCoV isolates. Interestingly, the avirulent strains UCD and 79-1683 (Pedersen *et al.*, 1981b; Pedersen, 1987), both designated as FECV, are more similar to virulent FIPV strains than to each other. In fact, the ORF7a/7b transcription units of FECV UCD and FIPV UCD3 are almost identical, displaying 99.6% nucleotide sequence identity.

Figure 3 shows an alignment of the deduced amino acid sequences and a comparison to the sequences published for TGEV and CCV. The 7a proteins are highly conserved among the FCoV strains, showing an overall sequence identity and sequence similarity of 87 and 90%,

A

	79-1146	79-1683	TN406	Dahlberg	Nor15/DF2	UCD1	UCD2	UCD3	UCD4	FECV UCD	Wellcome	CCV-K378
79-1683	93.2											
TN406	93.0	92.9										
Dahlberg	92.9	93.1	95.9									
Nor15/DF2	99.8	93.6	92.8	92.7								
UCD1	92.0	92.7	92.5	92.9	91.8							
UCD2	92.4	N.D.	92.1	91.6	92.3	92.9						
UCD3	91.9	92.4	92.3	92.5	91.7	95.1	93.5					
UCD4	90.5	91.8	89.3	89.4	90.3	90.2	89.7	89.8				
FECV UCD	92.2	92.8	92.6	92.8	92.0	95.4	93.7	99.6	90.1			
Wellcome	88.9	89.9	89.0	89.6	88.7	89.0	90.0	88.8	87.1	89.1		
CCV K378	78.3	78.3	78.9	78.6	78.1	79.0	80.2	79.5	76.7	79.7	78.7	
CCV Insavc-1	76.9	78.5	77.5	77.6	76.8	78.2	79.7	78.6	75.7	78.9	77.5	88.9

B

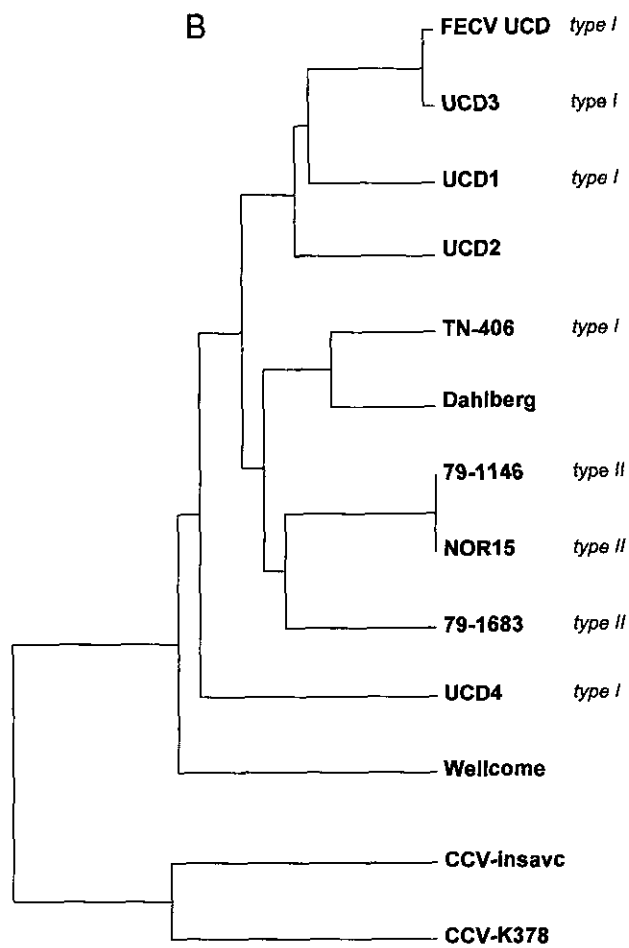

FIG. 2. (A) Percentages of nucleotide identity between the 7a/7b transcription unit and 3′ nontranslated region (NTR) of different feline and canine coronavirus isolates. (B) Dendrogram showing the relation of FCoV and CCV isolates in the 7a/7b transcription unit and 3′-NTR. The cDNA sequences were aligned using the multiple alignment software Pileup of the University of Wisconsin and the similarity scores obtained were used to create a dendrogram by the unweighed pair–group method using arithmetic averages according to Sneath and Sokal (1973). The distances along the vertical axes are proportional to the number of nucleotide substitutions.

respectively. The amino acid sequence of the 7b proteins is less well conserved, with sequence identities and similarities of only 65 and 76%, respectively. A potential glycosylation site at position 68 is present in all FCoV strains but not in the CCV strains.

Deletions in ORF7b of avirulent FCoV strains

For most FCoV strains, the RT–PCR yielded the expected products of 527, 485, and 513 bp when using primer sets 1, 2, or 3, respectively (Fig. 4A, lanes "NOR15"). However,

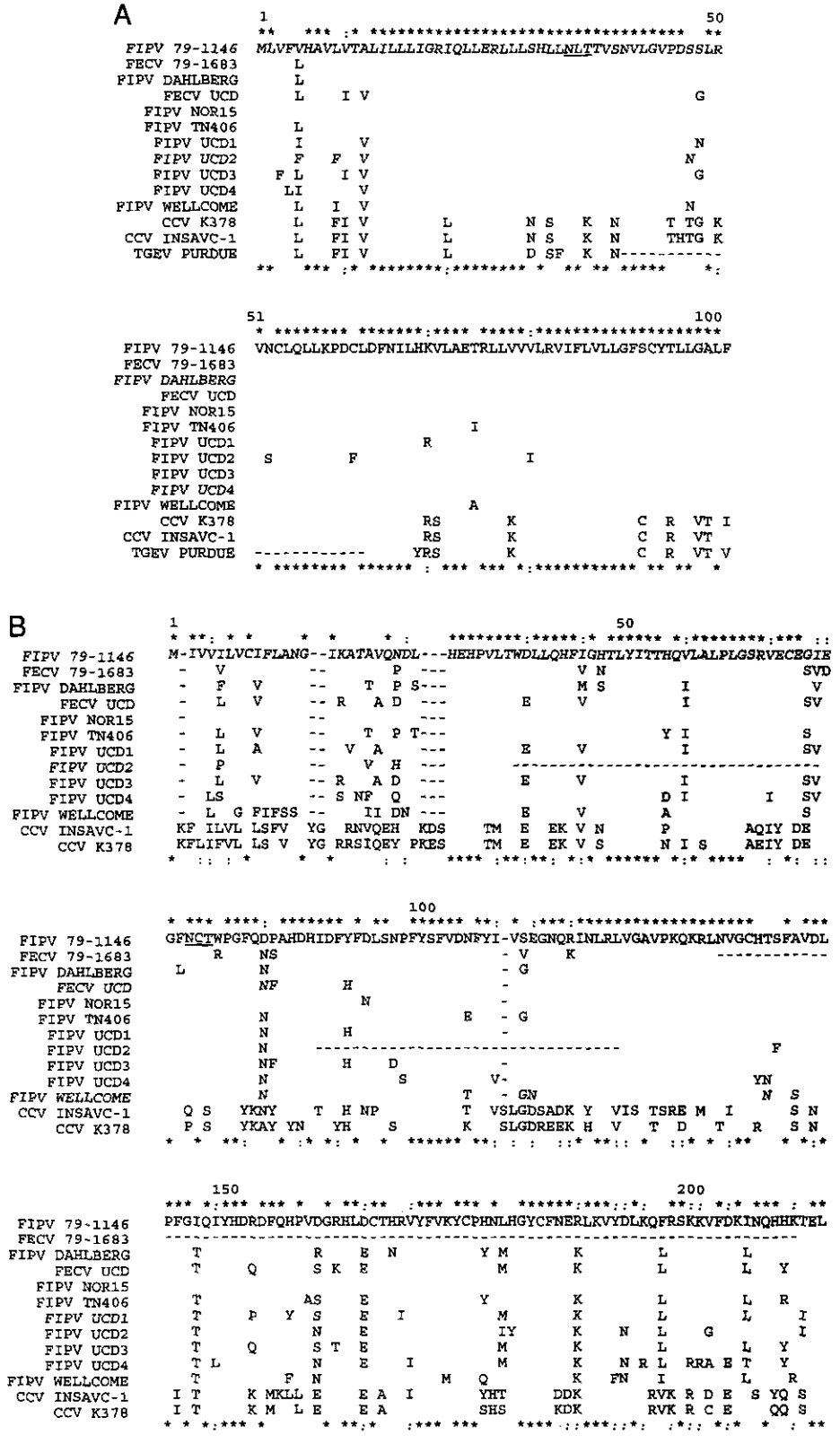

FIG. 3. Alignments of the amino acid sequences of the 7a (A) and 7b (B) proteins. Amino acid residues conserved between the different FCoVs and between FCoV, TGEV, and CCV are indicated by asterisks above and below the alignment, respectively. Conservative amino acid substitutions are indicated by (:). Gaps introduced in the sequence for maximum alignment are indicated by (—). Potential N-glycosylation sites in 7a and 7b are underlined. In the case of strains UCD2 and 79-1683, the frameshifts caused by the deletions in ORF7b were ignored; the amino acid sequences presented were deduced from the authentic 7b reading frame.

TABLE 2

Oligonucleotide Primers Used for RT–PCR and Cycle Sequence Analysis

Primer	Sequence (5′ to 3′)	Position[a]	Orientation
p178	GATGACACACAGGTTGAG	(−46)–(−29)	Sense
p202	CCTGCTATACATTGTTGGGTGC	275–296	Sense
p204	GCTCTTCCATTGTTGGCTCGTC	1136–1115	Antisense
p205	GGCAACCCGATGTTTAAAACTGG	932–954	Sense
p206	TCAGATTGGTTGGTGCTGTGCC	642–663	Sense
p211	CACTAGATCCAGACGTTAGCTC	1154–1133	Antisense
p261	GGGTATTGAAAGTCCCTGTCATG	759–737	Antisense
p267	GATCCAAGCGGTAGTGCTAAGA	480–459	Antisense
p287	ATTGTCGGCCGCATTATTTTAACATCATGATT	285–316	Sense
p299	GTGAGATTAAGCAGATGACTGAG	107–85	Antisense

[a] Numerical position on the genome of FIPV strain 79-1146 as counted from the initiation codon of ORF7a (*Fig. 1;* de Groot *et al.,* 1988).

in the case of the avirulent FIPV strain UCD2, primer set 2 yielded a product of only 295 bp (not shown), indicating that 190 nucleotides had been deleted from ORF7b. Surprisingly, in the case of FIPV strain TN406, primer set 2 not only yielded a product of the anticipated length but also a shorter one of 429 bp. Similarly, for FIPV strain UCD4 both a full-length product of 513 bp and another of only 456 bp were obtained when using primer set 3 (Fig. 4A). These findings indicated that the stocks of FIPV strains UCD4 and TN406 contained viruses with a full-length ORF7b as well as ORF7b deletion mutants. At low tissue culture passage levels of FIPV strain TN406 (TN406-LP), the parental virus and the ORF7b deletion mutant TN406-del appeared to be present in similar amounts. In high-passage stocks of TN406 (TN406-HP; Pedersen and Black, 1983), only TN406-del was detected (Fig. 4A).

The fact that UCD2, 79-1683, and high-passage TN406 are avirulent prompted us to study the structure of ORF7b in yet another attenuated FCoV strain, i.e., the NOR15-derived vaccine strain ts-DF2 (Christianson *et al.,* 1989; Gerber *et al.,* 1990). No RT–PCR products were obtained when using primer sets 2 (p202/p261) and 3 (p206/p211). Subsequent RT–PCR with primers p202 and p211 generated a 0.46-kb product instead of the expected 0.87-kb product (Fig. 4A). Sequence analysis showed that nucleotides 120–525 of ORF7b are deleted in ts-DF2. The structure of various ORF7b deletion mutants is shown schematically in Fig. 4B.

FCoV field strains contain an intact ORF7b

Our findings indicated that ORF7b deletion mutants readily arise during *in vitro* passage. We next asked whether on the other hand ORF7b is maintained during natural infection. Feces, plasma, ascitic fluid, or tissue samples were collected from 16 FCoV-infected cats (Table 3). Twelve of these had succumbed to natural FIP while 4 had experienced a subclinical FCoV infection and shed the virus in the feces (Herrewegh *et al.,* 1995; Herrewegh, unpublished observations). All samples contained viral RNA as shown by a FCoV-specific RT–PCR targeted to the 3′ nontranslated region (NTR) of the genome (Herrewegh *et al.,* 1995). The complete region containing ORF7b was amplified using a nested RT–PCR (Fig. 5A). The primers chosen bracket ORF7b and are from sequences conserved between FCoV, CCV, and TGEV. A 0.85-kb DNA was obtained for all FCoV isolates. These products comigrated with the ORF7b RT–PCR DNA obtained for FIPV strain NOR15 (not shown). To assay for the presence of small deletions, the PCR products were cut in an *Afl*III/*Xba*I/*Bcl*I triple digestion experiment. The *Afl*III site was conserved in all FCoV strains. The *Xba*I and *Bcl*I sites were absent in strains 8–14 and 15 and 16, respectively. Restriction fragments were separated in 2.5% agarose gels, again using strain NOR15-derived PCR DNA as a marker. No deletions were detected (Fig. 5B). Although the presence of nonsense codons or very small deletions (<10 bp) cannot be excluded, the data strongly suggest that ORF7b is maintained in all 16 field strains tested.

DISCUSSION

FECV and FIPV: Different species or virulence mutants?

FIP is a rare disease occurring only in a small proportion of the FCoV-seropositive cats. Yet, certain FIPV isolates such as 79-1146 are highly virulent and almost invariably cause FIP upon inoculation. To accommodate these findings, it was postulated that most cats become infected with FECV, an avirulent FIPV-related virus (Pedersen *et al.,* 1981b; Pedersen, 1983). This idea was welcomed to such an extent that FECV and FIPV have since been treated as different virus species.

A previous comparison of the 7a/7b transcription unit of FIPV strain 79-1146 and FECV strain 79-1683 already revealed an overall nucleotide sequence similarity of 93.5% (Vennema *et al.,* 1992b). It was noted, however, that in the FECV strain, ORF7b is truncated by a 238-nucleotide deletion. We now show that the other FECV isolate, UCD, contains an intact 7b gene, indicating that deletions in

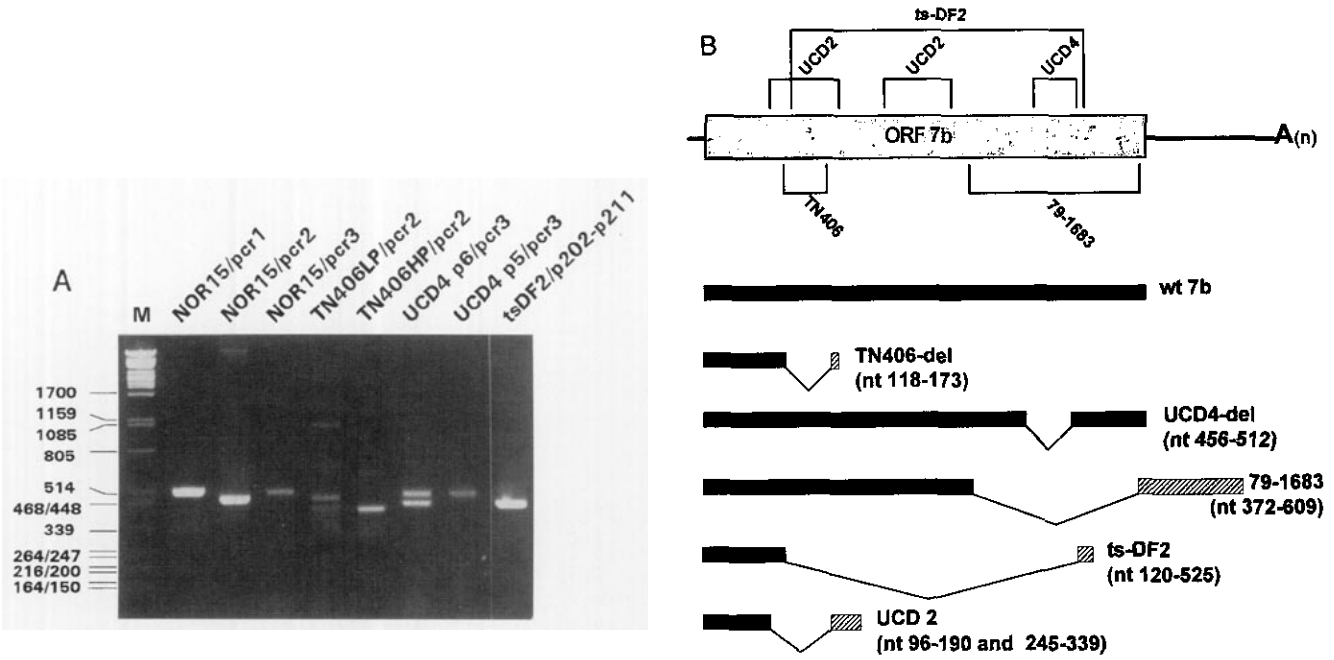

FIG. 4. Deletions in ORF7b of FCoV laboratory strains. (A) Strains TN406 and UCD4 were subjected to RT–PCR as in Fig. 1 using primer sets 2 and 3, respectively. Both low-passage (TN406LP) and high-passage (TN406HP) stocks of strain TN406 were studied. UCD4 was analyzed at passage levels 5 (p5) and 6 (p6). Also shown is the PCR product obtained for tsDF2 using primers p202 and p211. PCR products 1, 2, and 3 obtained for FIPV strain NOR15 were included as size markers. *Pst*I-digested phage lambda DNA served as a molecular weight marker (lane M). Sizes are given in base pairs. (B) Schematic representation depicting the structure of ORF7b in FCoV strains TN406-del, UCD4-del, 79-1683, ts-DF2, and UCD2. The upper panel shows ORF7b and the 3′-NTR. The sequences that have been deleted in the different FCoV strains are indicated by brackets. The lower panel shows the structure of the proteins encoded by the wild-type ORF7b (wt 7b) and by ORF7b deletion mutants. Black bars represent amino acid sequences encoded by ORF7b, and hedged bars represent non-7b sequences that are translated due to a frameshift. Also given are the nucleotides (nt) that have been deleted.

ORF7b are not a universal distinguishing property of feline enteric coronaviruses. In fact, the avirulent FECV strains UCD and 79-1683 are more similar to virulent FIPV strains than to each other, e.g., the nucleotide sequences of the ORF7a/7b transcription unit of FECV strain UCD and FIPV strain UCD3 are almost identical. Our findings support the notion that FECV and FIPV are not different species but merely virulence variants of the same virus (Barlough and Stoddart, 1990; Addie and Jarrett, 1992b).

TABLE 3

FCoV Field Isolates

No.[a]	Isolate	Specimen	Clinical status[b]
1	FIPV UCD5	Omentum	FIP
2	FIPV UCD6	Omentum	FIP
3	G2010	Ascites	FIP
4	Riem	Feces	Healthy
5	F297	Ascites	FIP
6	D2461	Spleen	FIP
7	D2490	Ascites	FIP
8	SBB	Feces	Healthy
9	F461	Ascites	FIP
10	6855	Feces	Healthy
11	6937	Feces	Healthy
12	D2432	Kidney	FIP
13	D2494	Ascites	FIP
14	D2706	Ascites	FIP
15	G2022	Ascites	FIP
16	G2023	Ascites	FIP

[a] Numbers correspond to those in *Fig. 5B*.
[b] Clinical status of the host at the time of sampling.

The epidemiological data can be explained by assuming that FCoVs of moderate virulence predominate in the field, i.e., highly virulent viruses may be just as rare as completely avirulent ones (Pedersen and Floyd, 1985; Pedersen, 1987). In this scenario, factors such as virus dose, concomitant infections, stress, and possibly the genetic background of the host also determine whether upon an FCoV infection FIP ensues. An alternative explanation is that most FCoVs are avirulent and that highly virulent mutants arise spontaneously in individual hosts. In this latter case it must be assumed that the spread of such virulent mutants from one host to another is limited since true outbreaks of FIP in domestic cats are rarely reported.

Type I and II FCoVs cannot be distinguished in the ORF7a/7b transcription unit

From the comparison of the nucleotide sequences of the ORF7a/7b transcription unit, the FCoVs emerge as a rather homogeneous group. Type I and II FCoVs cannot be distinguished in this part of the genome and are clearly more related to each other than to the CCV strains sequenced thus far. These findings seem to conflict with observations

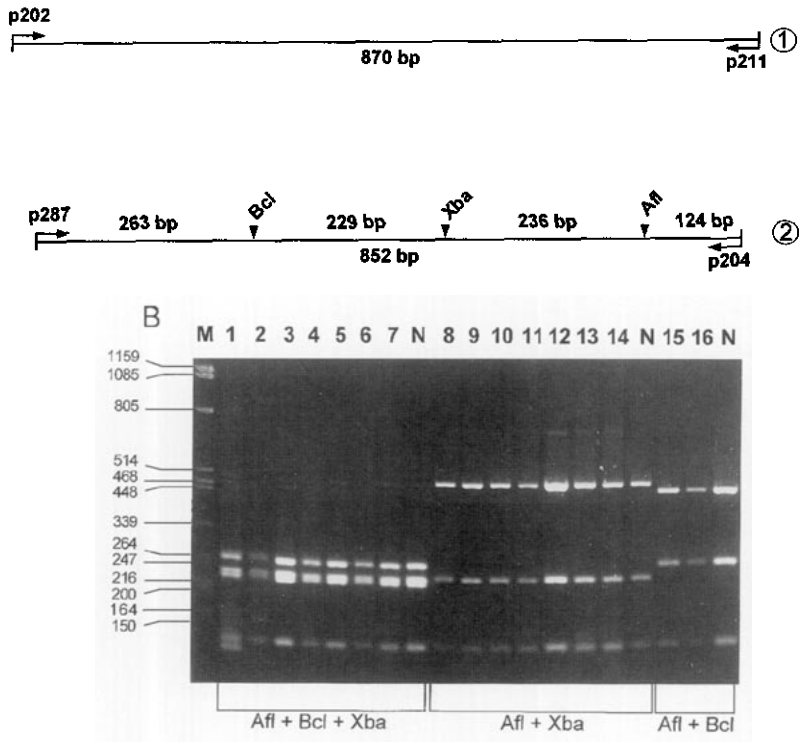

FIG. 5. RT–PCR analysis of ORF7b of naturally occurring FCoVs. (A) An outline of the nested RT–PCR amplification of the complete ORF7b gene. The ORF7a/7b transcription unit is depicted schematically. In the case of an intact ORF7b, a 870-bp product is generated by a first RT–PCR with primers p202 and p211 (1). A second PCR with primers p287 and p204 will generate a product of 852 bp (2). Also indicated are the sites of the restriction enzymes *Bcl*I, *Xba*I, and *Afl*III and the sizes of the restriction fragments. (B) RNA was extracted from various tissue samples, ascitic fluid, or feces from 16 cats with a natural FCoV infection (Table 3). The RNA was then subjected to a nested RT–PCR as in A. To test for small deletions, the RT–PCR products were cut with *Afl*III+*Bcl*I+*Xba*I. Restriction fragments were analyzed in 2.5% agarose gels (lanes 1–16). *Pst*I-digested lambda DNA was used as a molecular weight marker (lane M). For comparison, the RT–PCR product of FCoV strain NOR15 digested with either *Afl*III+*Bcl*I+*Xba*I, *Afl*III+*Xba*I, or *Afl*III+*Bcl*I is also shown (lanes N).

made by others (Pedersen *et al.*, 1984a; Hohdatsu *et al.*, 1991a), who found that type II FCoVs are antigenically more similar to CCV than to the type I FCoVs. It would appear, however, that the antigenic differences between type I and II viruses are mainly restricted to the spike protein (Fiscus and Teramoto, 1987; Hohdatsu *et al.*, 1991b). Indeed, several S-specific MAbs were isolated that recognize CCV and type II FCoVs but not type I FCoVs (Hohdatsu *et al.*, 1991a; Corapi, personal communication). Very recently, these immunological findings were confirmed by sequence analysis. The spike genes of the type II FCoV strains 79-1146 and 79-1683 bear a much greater resemblance to those of TGEV and CCV than to the spike genes of the Japanese type I isolate KU-2 (Motokawa *et al.*, 1995) and the American type I isolate UCD3 (Vennema *et al.*, 1995).

How can these observations be reconciled with our present findings? During coronavirus replication homologous RNA recombination occurs at a high frequency both in tissue culture cells and *in vivo* (for a review see Lai, 1992). It is therefore quite conceivable that the type II FCoV strains have arisen by an RNA recombination event between a type I FCoV and CCV. We have recently sequenced the region between the S and N genes for several type I and II FCoVs. These studies revealed that the type II strains 79-1146, UCD6 (Vennema *et al.*, manuscript in preparation), and 79-1683 (Herrewegh *et al.*, manuscript in preparation) are indeed recombinant viruses.

Deletions in ORF7b correlate with decreased virulence

ORF7b encodes a nonstructural, secretory glycoprotein, gp7b, that is dispensable for viral replication in tissue culture (Vennema *et al.*, 1992a). Furthermore, gp7b also appears to be dispensable for the infection of the natural host: the avirulent FCoV strain FECV 79-1683 can infect cats (Pedersen *et al.*, 1984b). Yet, ORF7b is present — at least in low-passage stocks — in most FCoV lab-

oratory strains analyzed thus far. Also, no deletions were detected by RT–PCR analysis in the ORF7b of 16 naturally occurring FCoVs. Thus, although readily lost during *in vitro* growth, ORF7b seems to provide a distinct selective advantage during the natural infection. Interestingly, all 4 FCoV strains with deletions in ORF7b (79-1683, TN406-HP, UCD2, and ts-DF2) are avirulent (Pedersen, 1983, 1987; Pedersen and Black, 1983; Pedersen and Floyd, 1985; Christianson *et al.*, 1989). It is quite likely that for these viruses the ORF7b deletions contribute to the loss of virulence. Whether these deletions are the only or even the most important cause of attenuation is unknown. Changes may also have occurred in other regions of the genome. For instance, FECV strain 79-1683 has at least one additional deletion of 109 nt in ORF3b-c compared to the virulent strain 79-1146 (Herrewegh, unpublished observations). The precise function of gp7b remains enigmatic. Extensive searches in protein databases have not yielded significant homologies (Alexander Gorbalenya, personal communication). Presumably, gp7b exerts its function in the extracellular medium, perhaps as a virus-encoded mediator of the inflammatory or immune reaction. Such proteins have been described for several large DNA viruses, e.g., herpesviruses, poxviruses, and adenoviruses (Wold and Gooding, 1991; Gooding, 1992; Smith, 1993). The complexity of the coronaviral genome, the largest RNA genome known, would certainly allow for the presence of accessory genes.

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