Cloning and sequence determination of the feline calicivirus strain F9.

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Feline Caliciviral infection is typically associated with mild depression, sneezing, ocular and nasal discharges, and often mouth ulceration. However, isolates vary in their pathogenicity. Some isolates reproducibly cause a more severe syndrome; some induce oral or nasal ulceration alone; some induce an interstitial pneumonia and some appear to be apathogenic[1-3]. Infection with feline calicivirus (FCV) is common despite over ten years of vaccination. Thus, FCV has strategies which ensure its continuing importance as a pathogen of cat.

The molecular biology of calicivirus, and in particular FCV, is not well understood. FCV are small, non-enveloped viruses which contain a positive stranded RNA genome. Two 'strains' have now been partially cloned and sequenced; one in the USA (CF1/68 FIV (FCV)[4-6]; and another (vaccine strain F9)[3] in the UK. The UK vaccine strain F9 that we have cloned may be smaller as we estimate its size at 7kb compared to the 8kb described for the American isolate[4].

To date, we have sequenced 5284bp of cDNA from the polyadenylated 3'-end of the virus genome towards the 5'-end. This region contains four candidate open reading frames (ORFs) predicted by computer analysis, which we designate from the 3'-end. Three are complete and comprise ORF1 of 246, ORF2 of 2013 and ORF3 of 318 nucleotides. The fourth, ORF4, present in the same frame as ORF2 extends beyond the sequenced region towards the 5' end of the viral genome and is at least 3000bp in size.

By making fusion proteins, we have previously shown that ORF2 encodes the precursor of the capsid protein, 671 amino acids long and some 73,441 in molecular weight, which loses 124 residues at the N-terminus during maturation[8]. Although the coding assignment for ORF3 is not yet certain it appears to correspond to the putative non-structural gene identified previously[5].

This sequence also overlaps that already published for strain CF1/68 FIV (FCV)[5] and also a second sequence deposited with the EMBL database from the 3' end of this FCV strain[6]. The sequence homology in the area of overlap averages 80% as determined by the DNAsis Dotplot program. This sequence contains the same pattern of four potential ORFs regions as that described for strain F9 except that ORFs 1 and 2 are in the same reading frame. The extent of nucleotide identity between the structural (87%) and probable non-structural genes (78.8%) in the areas of overlaps are very similar[8]. The predicted protein sequences are even more closely related, 95.3% identity for ORF 1, 89.71% for ORF2, and 91.4% in the region of overlaps between ORF3 from both strains.

The capsid sequence derived from strain F9 and that reported for strain CF1/68 FIV reveals that this protein contains both highly conserved and more variable regions[8]. The latter may be responsible for the observed antigenic and pathogenic divergence between strains of FCV. We are at present obtaining the remaining sequence of the F9 strain to study the genome and its expression in more detail. Furthermore, we are comparing the capsid gene sequence in various FCV isolates to identify the factors responsible for strain variation. This should enable us to devise a broad spectrum vaccine having high efficacy.

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