The S gene of canine coronavirus, strain UCD-1, is more closely related to the S gene of transmissible gastroenteritis virus than to that of feline infectious peritonitis virus

Ronald D. Wesley *,1

Virology Swine Research Unit, National Animal Disease Center, USDA, Agricultural Research Service, P.O. Box 70, 2300 Dayton Avenue, Ames, IA 50010, USA

Abstract

To gain insight into the genetic relationships among six canine coronavirus (CCV) strains, the variable region of the spike (S) protein gene was sequenced. The CCV strains were: two ATCC reference strains, the Insavc-1 vaccine strain, the National Veterinary Services Laboratories (Ames, IA) challenge strain, and two California field isolates (UCD-1 and UCD-2) from the 1970s. All six strains, downstream of the nucleocapsid (N) protein gene, had sufficient size for an ORF 7b, and thus, none were transmissible gastroenteritis virus (TGEV)-like since TGEV lacks ORF 7b. By sequence analysis of the variable domain at the 5′ end of the S gene, five of the six CCV strains had a high degree of identity with feline infectious peritonitis virus (FIPV). However, one CCV field isolate (UCD-1) was different and had a high degree of identity with the 5′ end of the TGEV S gene. This suggests that RNA recombination occurred at this site between antigenically related coronaviruses. The low passage field isolates, UCD-1 and UCD-2, varied in their initial infectivity for swine testicular cells suggesting that sequence differences in the variable domain of the S gene may account for biological variation among CCVs. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Coronaviruses infect a number of different vertebrate species and cause economically important diseases in people, pets, livestock and poultry. These coronaviruses are classified into three distinct groups. One tightly clustered subgroup based on phylogenetic analysis and antigenic cross reactivity contains canine coronavirus (CCV), transmissible gastroenteritis virus (TGEV), and feline

* Tel.: + 1-515-239-8358; fax: + 1-515-239-8384.

E-mail address: rwesley@nadc.ars.usda.gov (R.D. Wesley)

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coronaviruses (FCoVs) (Pedersen et al., 1978; Siddell, 1995). The FCoVs are divided into two distinct serological groups referred to as types I and II and either type can manifest itself in cats as feline infectious peritonitis (FIP), feline enteric disease, or a subclinical infection (Hohdatsu et al., 1991; De Groot and Horzinek, 1995).

Sequencing of the spike (S) protein gene of TGEV and feline infectious peritonitis virus (FIPV) has shown a high degree of identity except for the first 274 amino acids at the N-terminus of the protein where the sequences diverge dramatically (Jacobs et al., 1987). Likewise, S gene sequencing of CCV strains has shown that they have a closer relationship to type II FCoVs than to either type I FCoVs or to TGEV (Horsburgh et al., 1992; Wesseling et al., 1994; Horsburgh and Brown, 1995). In fact, the type II FCoVs apparently evolved by a double recombination between type I FCoVs and CCV (Herrewegh et al., 1998). Another genetic region that distinguishes among these coronaviruses is at the very 3′ end of the genome. Both CCV and FIPV have open reading frames (ORFs) 7a and 7b, and thus, are different from TGEV which lacks ORF 7b and has a single smaller ORF 7 because of a 69 nucleotide deletion (De Groot et al., 1988; De Groot and Horzinek, 1995).

In vitro growth properties also distinguish these coronaviruses. FCoVs are very difficult to grow in cell culture, at least on primary isolation, and replicate only in cells derived from their natural host (De Groot and Horzinek, 1995). Transmissible gastroenteritis virus is also difficult to grow on initial isolation but replicates both in canine and porcine cell cultures (Welter, 1965; Ruitenberg et al., 1969; Woods and Wesley, 1988). CCV can be grown in canine or feline cells (Crandell et al., 1973; Binn et al., 1980) but does not grow in swine thyroid or swine testicular cells (Reynolds et al., 1980; Woods and Wesley 1988).

The CCVs have shown cross-species infectivity. However, results from these studies were contradictory. One CCV strain, 1-71, apparently was unable to infect neonatal or nursery-age pigs (Binn et al., 1974). In contrast, neonatal pigs were infected with another CCV strain, UCD-1, as shown by positive immunofluorescence in cells of the crypt epithelium (Woods et al., 1981). Moreover, CCV strain UCD-1 infected older pigs (sows and gilts) as shown by the presence of neutralizing antibody (Woods and Wesley, 1986) whereas sows exposed to FIPV did not seroconvert (Woods and Pedersen, 1979). In another study, an old National Veterinary Services Laboratories (NVSL) CCV challenge strain (DK-1) was transmitted to pigs that were placed in contact with CCV-infected dogs (Woods and Wesley, 1992).

Because of these possible strain differences, the N-terminal half (approximately 2.4 kb) of the CCV S gene that includes the variable domain was sequenced. For this study, two CCV field strains of US origin, two CCV reference strains from the American Type Culture Collection (ATCC) and the current USDA, APHIS, NVSL challenge strain were sequenced and compared with S gene sequences of the CCV Insavc-1 strain (Horsburgh et al., 1992).

2. Materials and methods

2.1. Cells

Fetal cat (FC) cells (Woods, 1982) were used to replicate CCV strains and swine testicular (ST) cells (McClurkin and Norman, 1966) were used to replicate TGEV strains. Both the FC and ST cell lines were grown in modified Eagle's minimum essential medium (MEM, Gibco BRL, Gaithersburg, MD) supplemented with fetal bovine serum (FBS, 10%), sodium bicarbonate (0.22%), lactalbumin hydrolysate (0.25%), sodium pyruvate (1 ×), and gentamicin sulfate (50 µg/ml).

2.2. Viruses

CCV strains UCD-1 (ZacMoore) and UCD-2 (Yaskulski) and FIPV strain UCD-1 were kindly provided by Dr N. Pedersen, University of California at Davis. The CCV strains were originally isolated from dogs with fatal gastroenteritis at different kennels in northern California. These California field isolates were grown at a low number of passages in a feline cell line (fcwf cells, Horzinek et al., 1982). CCV strain Insavc-1

(kindly provided by Intervet, Millsboro, DE) was originally isolated from feces of a young dog with severe gastroenteritis. The virus was isolated and plaque purified in A-72 cells. Two CCV strains, 1-71 and TN449, were obtained from the ATCC (Rockville, MD). Strain 1-71 was isolated from feces of a dog with diarrhea and passaged seven times in primary dog kidney cells and twice in A-72 cells. Strain TN449 was isolated from a dog with severe gastroenteritis and passaged in CRFK cells until attenuated. The CCV challenge virus (95-08) is a field isolate obtained from the USDA, APHIS, NVSL, Ames, IA.

The TGEV strains used were the Miller strain (Wesley et al., 1989) which was plaque purified and the high passage Purdue strain (P-115e) kindly provided by Dr L. Saif, Ohio Agricultural Research and Development Center, Wooster, OH.

2.3. RNA Extraction and RT-PCR

A confluent cell culture in a 75 cm² flask (Becton Dickinson, Franklin Lakes, NJ) was infected with the appropriate coronavirus. When cytopathic effect was extensive, the media in the flask was removed and RNA was prepared with the RNeasy total RNA kit using a QIAshredder (Qiagen, Chatsworth, CA).

A single-tube Titan™ reverse transcriptase-polymerase chain reaction (RT-PCR) system was used to amplify sequences (Boehringer Mannheim, Indianapolis, IN). The reactions were carried out in a final volume of 50 µl containing 10 pmol of each primer, 10 mm of each dNTP, $1 \times$ RT-PCR buffer with Mg^{2+}, 0.3 U RNase inhibitor (5′–3′, Boulder, CO), 1 µl Expand™ High Fidelity DNA polymerases with AMV reverse transcriptase, and 0.5 µg RNA. Primers were designed on either side of ORFs 7a/7b and synthesized (Integrated DNA Technologies, Coralville, IA). The sense primer, 5′-GTGTTT-GATGACACACAGGTTGAG-3′ (N3SN), and anti-sense primer, 5′-GCTTACCATTCTGTA-CAAGAGTAG-3′ (R3AS), were located at nucleotide positions 8301 and 9359, respectively, at the 3′ end of the Insavc-1 genome (Horsburgh et al., 1992). The primers were selected for high melting temperatures and because they generated ampli-

cons for all of the coronavirus strains. Reaction conditions for RT-PCR were to incubate at 50°C for 30 min followed by 95°C for 5 min. This incubation was followed by 25 cycles of denaturation (95°C for 30 s), annealing (65°C for 30 s) and extension (68°C for 1 min). A final extension at 68°C for 7 min was carried out before holding the samples at 4°C.

2.4. DNA sequencing

Prior to direct sequencing, RT-PCR amplicons were purified with Geneclean spin columns (Bio 101, Vista, CA). Cycle sequencing using Taq polymerase and fluorescently labelled dideoxynucleotides (PE Applied Biosystems, Foster City, CA) was performed on both strands by an Applied Biosystems 377 automated sequencer. For a final consensus sequence, all regions were sequenced at least three times. Eleven sense primers and 12 anti-sense primers were used for sequencing the five CCV strains.

The nucleotide sequences of the five strains are deposited in GenBank with accession numbers AF116244 to AF116248.

2.5. Adaptation of CCV to ST cells

A separate 8-chamber slide (Nunc, Naperville, IL) with confluent ST cells was used for each CCV strain. A 10-fold dilution series in Eagle's MEM with 2% FBS was prepared for CCV strains UCD-1 (2.7×10^6 PFU/ml) and UCD-2 (1.8×10^6 PFU/ml). Four wells in a row were infected with 0.4 ml of different virus dilutions from full-strength to 10^{-3} and the four adjacent wells with fresh 2% media served as uninfected control ST cells. The chamber slides were incubated at 37°C for 4–5 days in a CO_2 incubator, and then, supernatant from each well was blind passed to the corresponding well of a new 8-chamber slide with confluent ST cells. The first slide was fixed and the second slide was incubated for another 4 days. After incubation, the supernatant from the second slide was removed and stored at -70°C, this slide was fixed and all slides were processed for CCV antigen detection.

2.6. Immunofluorescence

Each well was rinsed with phosphate buffered saline (PBS) and fixed with methanol–acetone (1:1) for 10 min at room temperature. The slides were then air dried and processing continued or stored for later processing. Slides were rinsed 3 × with PBS and then washed 1 × with PBS containing 0.25% bovine serum albumin (BSA) for 10 min at room temperature. Anti-TGEV hyperimmune direct conjugate (100 μl) was added to each well and incubated for 1 h at room temperature in a humidified chamber. The conjugate was flicked off and the slides were rinsed 3 × in PBS followed by 2 × in double-distilled H$_2$O. Air dried slides were examined and photographed with a fluorescence microscope.

2.7. Computer analysis

Primers for sequencing and RT-PCR were selected using Primer Designer (versions 1.01 and 2.01, Scientific and Educational Software, Durham, NC). Sequence analysis and alignments were done with GeneWorks version 2.5.1 (Intelligenetics, Mountain View, CA). Percent identity between sequence pairs was determined using MegAlign version 4.0 (DNASTAR, Madison, WI).

3. Results

3.1. RT-PCR analysis of the ORF 7a/7b region

Five CCV field strains and the Insavc-1 vaccine strain were analyzed by RT-PCR to determine if they were large enough downstream of the nucleocapsid gene to contain an ORF 7b and thus differ from TGEV strains (Fig. 1). All six CCV strains produced DNA amplicons of the same size (1082 bp). The CCV amplicons were slightly larger than the DNA amplicon generated by the FIPV strain (1061 bp) and considerably larger than the 367 bp amplicons produced by two TGEV strains. This confirmed that the six CCV strains originally isolated from dogs were similar at the 3′ end of the genome because they all were large enough for an ORF 7a/7b region.

3.2. N-terminal sequencing of the S-gene

Fig. 2 shows the deduced amino acid sequences for the N-terminal half of the S-gene for CCV strains UCD-1, UCD-2, TN449, 1-71, NVSL and their alignment with the previously sequenced reference strains Insavc-1, FIPV 79-1146 and TGEV Miller strain. Downstream of UCD-1 strain amino acid 273, all six CCV strains had a high degree of identity (91–99%). However, between amino acid residues 1 and 273, the UCD-1 strain was distinctly different from the other five CCV strains as shown by the percent identity between each sequence pair (Table 1). UCD-1 had only 19–20% identity with the other CCV strains in this region while the others were 79–100% identical. Moreover, in this N-terminal region upstream of residue 273, UCD-1 strain had 86% identity to the same region of the TGEV S-gene (strains Miller and Purdue) but only a 24% identity in this
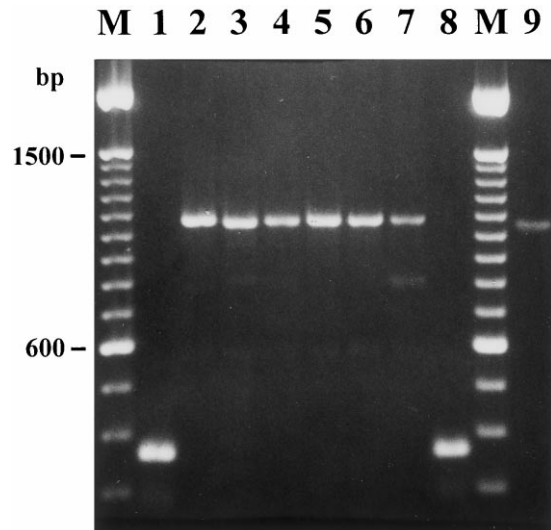
Fig. 1. Comparison of DNA amplicons from canine coronaviruses (CCVs), transmissible gastroenteritis viruses (TGEVs) and feline infectious peritonitis virus (FIPV). The DNA amplicons were produced by reverse transcriptase-polymerase chain reaction (RT-PCR) with primers flanking the open reading frames (ORFs) 7a/7b region and separated on a 1.5% agarose gel. The RNA templates were from: (1) TGEV Miller strain, (2) CCV UCD-1, (3) CCV UCD-2, (4) CCV TN449, (5) CCV 1-71, (6) CCV NVSL, (7) CCV Insavc-1, (8) TGEV Purdue strain, (9) FIPV UCD-1, and (M) 100 bp DNA ladder (Gibco BRL).

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Consensus    MIVL..CLLL ..SY..V..T .NNDC.QVNV TQLPGNEN.I .DFLF..FKE EGSVVVGGYY PT-EVWYNCS R.A.TTAY.. F.NIHAFYFD MEAMENSTGN    100

FIPV         ....VT.... LC..HT.LS. T..E.I.... ...A...L. R....SN... .......... .......... .T.R..FQY .N.......V ..........     99
Insavc-1     ....TL..F. F-L.SS.SC. S...V.... .......I. K....QN... ...L...... TTQQ...KY .S........ .......... ..........      98
1-71         ....IL.... F-..NS.IC. S...V.... .......I. K....HT... .......... .S.T....KD .S........ .......... ..........      98
NVSL         ....IL.... F-..NS.IC. S...V.... .......I. K....HT... .......... .S.T....KD .S........ .......... ..........      98
TN449        ....VT.I.. LC..HT.LS. A...R.... .......V. R....QS... .......... .......... .T.T....QY .N........ .......... ..........      99
UCD-2        ....LT.I.. LC..QT.SS. S.S..R.... .......YI. R....SS.T. .......... .......... .T.P....EY .S........ .......... ..........      99
UCD-1        .KL.FVV.VV LP.IYGDNFP CSKFLNRTIG NHWNLI..FL LNYSIR-LPP NSD..L.D.F .VQP.F..I .NNNNSL.VT ME.FK.L.W. Y-.T..I.SD      98
TGEV         .KK.FVV.VV MPLIYGDNFP CSKLTNRTIG NHWNLI.TFL LNYSSR-LSP NSD..L.D.F .VQP.F..I .NNSNDL.VT LE.LK.L.W. Y-.T..I.L.      98


Consensus    ARGKPLLVHV HG.PVSIIIY ISAYRDDVQ. RPLLKHGL.C ITK...IDYN .FTS.QW.SI C.G.DR-KIP FSVIPTDNGT KIYGLEWNDD .VTAYISG.S    200

FIPV         .......F.. ..E..V..- -........Q ........V. ...NRH.N.E Q...N..N.T .T.A...... .......... .......... F.......R.    196
Insavc-1     .......... ..N.....V. .........F ........L. ...NDTV... S..IN..RD. .L.D...... ...V...... .LF....... Y......DE.    197
1-71         .......... ..D....... .........G ........L. ...NKI.... T..A..SA. .L.D...... .......... .F........ Y......DR.    197
NVSL         .......... ..D....... .........G ........L. ...NKI.... T..A..SA. .L.D...... .......... .F........ Y......DR.    197
TN449        .......F.. ..D..V... .........H ........V. ...SRN.... T...S..N.. .T.N...... .......... .......E F......H.    198
UCD-2        .......... ..E....... .........N ........V. ...TRT.... A...S..N.. .T.N.....S .......... .......E F......R.    198
UCD-1        H.QR-.H.V. K.K.Y..TVT TTRNF.AAEG -AIIC---I. KGSPPHTTTG NLDC-N.G.D .RLNHKFP.C P.NSQANC.N ML...Q.FT. E.V..LH.AI    192
TGEV         HKQR-.N.V. N.Y.Y..TVT TTRNFNSAEG -AIIC---I. KGSPPTTTTE SSLTCN.G.E .RLNHKFP.C P.NSEANC.N ML...Q.FA. A.V..LH.A.    193


Consensus    Y.LNINNNWF NNVTLLYSRS STATWQ.SAA YV--YQGVSN FTYYKLNNTN GLK.YE.CED YEYCTGYATN VFAPT.GGYI PDGFSFNNWF LLTNSSTFVS    300

FIPV         .H....T... .......... ....EY... ..A....... .......... ...T..L... ..H....... ....S..... .......... F.......      294
Insavc-1     HR........ .......T ......H... .......... .....K.A ..S..L... .......... ....S..... .......... M.........      295
1-71         HH........ ....I..... ......K... .......... .....NKI... ..S..L... .......... ....V..... .......... M.........      295
NVSL         HH........ ....I..... ......K... .......... .....NKI... ..S..L... .......... ....V..... .......... M.........      295
TN449        .NW....... .......... ......H... .......... .......... ...T..F... ......H... .....V.... .......... ..........      296
UCD-2        .K........ .......... ......N... .......... .......... ...T..F... .......... .......... .......... ..........      296
UCD-1        .RISFE.K.. GT...GDM.A T.LQTAGALV DLWWFNP.YD V...RV..K. .TTIVSN.T. --Q.AS.VD. ..TTQP..L. .SD....... ......V..    290
TGEV         .RISFE.Q.S GT...GDM.A T.LETAGTLV DLWWFNP.YD VS..RV..K. .TTVVSN.T. --Q.AS.VA. ..TTQP..F. .SD....... .......L..    291


Consensus    GRFVTNQPLL VNCLWPVPSF GVAAQEFCFE GAQFSQCNGV SLNNTVDVIR FNLNFT.DVQ SGMGATVFSL NTTGGVILEI SCYNDTVSES SFYSYGEIPF    400

FIPV         .......... I......... .......... .......... .......A... .......... .......... ..S....... .S........      394
Insavc-1     .......... .......... .......... .......... ....T..... .......... .......... .......... .......... ..........      395
1-71         .......... .......... .......... .......... ....T..... .......... .......... .......... .......... ......S.      395
NVSL         .......... .......... .......... .......... ....T..... ...IF-.... .......... .......... .......... ......S.      394
TN449        .......... .......... .......M.. .......... .......A... .......... .......... ..K....... .......... ..........      396
UCD-2        .......... S......... .......... .......... .......A... .......... .......... .......... .......L.. ..........      396
UCD-1        .KL..R...V .......... KE..ST.... .G.D....A V......... .......A... .......... ....I..... .......D...      390
TGEV         .KL..K.... .......... EE..STL... .G.D....P V......... ....TN... ..K....... .......T... ........D. ..S....M..    391


Consensus    G.TDGPRYCY VLYNGTALKY LGTLPPSVKE IAISKWGHFY INGYNFFSTF PIDCISFNLT TG.SGAFWTI AYTSYTEALV QVENTAIKKV TYCNSHINNI    500

FIPV         .I........ .......... .......... .......... .......G... .......V.. .......... .......... .......N..      494
Insavc-1     .V........ .......... .......... .......... .......A... ..A....... .......... .......... .......... ..........      495
1-71         .V........ A......... .......... .......... .......D... .......... ...D...... .......... .......... ..........      495
NVSL         .V........ A......... .......... .......... .......D... .......... ...D...... .......... .......... ..........      494
TN449        .I........ .......... .......... .......... .......A... .......... .......... .......... .......... ..........      496
UCD-2        .I........ .......... .......... .......... .......A... .......... .......... .......... .......... ..........      496
UCD-1        .I........ ....D..... .......... .......... .......... ..A....... .......... .......... .......... ..........      490
TGEV         .V........ .......... .......... .......... .......D.DV... .......... .......T... ....YV...    491


Consensus    KCSQLTANL. NGFYPVASSE VGLVNKSVVL LPSF..HT.V NITIDLGMKR SGYGQPIAST LSNITLPMQD NNTDVYCIRS NQFSVYVHST CKSSLWDN.F    600

FIPV         ........N .......... ...F...... ...FTY.A.. ........L .......... .......... .......... .......... ......I.      594
Insavc-1     ........Q E......... ...YS..S.. .......... .V-TVT..P .......... ...I...... .......... .......... ......N.      594
1-71         ........Q .......... ...YS..S.. .......... .......... .......... .......... .......... .......... ....DV.      595
NVSL         ........Q .......... ...YS..S.. .......... .......... .......... .......... .......... .......... ....DV.      594
TN449        ........N .......... ...T.FA.AF .......... .......... .......... .......... ....V..... .......... ...S..I.      596
UCD-2        ........N .......... ...FA..T.. .......... .......... .......... ....L..... .......... ....I ...S..I.      596
UCD-1        ........Q .......... ...FA..T.. .......... .......... .......P... .......... .......I.... .......V.      590
TGEV         ........N ......S... .......... ...YT..I. ....G..... .......... .......... .......D... .......... ....V.      591


Consensus    N.DCTDVL.A TAVIKTGTCP FSFDKLNNYL TFNKFCLSLS PVGANCKFDV AARTRTNEQV VRSLYVIYEE GDNIVGVPSD NSGLHDLSVL HLDSCTDYNI    700

FIPV         .Q.....E.. .......... .......... .......... .......... .......... .......... .......... .......... ..........      694
Insavc-1     .SA.....D. .......... .......... ........N .......L.. .......... FG........ .......... .......... ..........      694
1-71         .S......Y. .......... .......... ........N .......... .......... .......... .......... .......... ..........      695
NVSL         .S......Y. .......... .......... ........N .......... .......... .......... ...A...... .......... ..........      694
TN449        .Q.....E.. .......... .......... .......... .......... ....H..... .......... .......... .......... ..........      696
UCD-2        .Q.....E.. .......... .......... .......... .......... .......... ....L..... .......... .......... ..........      696
UCD-1        .Q.....E.. ....I..... .......... .......... .......... .......... .......... .......... .......... ..........      690
TGEV         KRN.....D. .......... .......... .......... .......... .......... ....D..... .......... .......... ..........      691


Consensus    YGRTGVGIIR QTNSTLLSGL YYTSLSGDLL GFKNVSDGVI YSVTPCDVSA QAAVIDGAIV GAMTSINSEL LGLTHWTTTP NFYYYSIYNY TNERTRGTAI    800

FIPV         .......... R......... .......... .......... .......... .......... .......... .......... .......S.. ..........      794
Insavc-1     .......... K......... .......... .......V.. .......... .......... .......... .......... ..VMN.....      794
1-71         .......... .......... .......... .......... .......... .......... .......... .......... ........-- ..........      793
NVSL         .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........      794
TN449        .......... .......... .......... .......V.. .......... .......... .......... .......... .......... ..........      796
UCD-2        .......... .......... .......... .......... .......... .......... .......... .---------- ----------      777
UCD-1        ......V.. .......... .......... .......... .......... .......... .......... .......... .......... ..........      790
TGEV         .......... R......... .......... .......... .......... .......T.. .I........ .......... ..D.......      791


Consensus    DSNDVDCEP. ITYSNIGVC    819

FIPV         .........V .........    813
Insavc-1     .-..I...I .........    812
1-71         ---------- ---------    793
NVSL         .........I .........    813
TN449        .........I ..A.....    815
UCD-2        ---------- ---------    777
UCD-1        .........I .......--    807
TGEV         .........V .........    810
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Fig. 2. Alignment of deduced amino acid sequences of the S gene of six canine coronaviruses (CCVs), feline infectious peritonitis virus (FIPV) 79-1146, transmissible gastroenteritis virus (TGEV) Miller strain and the consensus sequence. Amino acids 1–273 of CCV UCD-1 are similar to those of TGEV but divergent from N-terminal sequences of the other five CCVs and FIPV.

Table 1
Sequence pair distances for S gene amino acids 1–273 of six canine coronavirus (CCV) strains

Divergence	Percent identity							
	1	2	3	4	5	6		
1	–	79.1	87.8	87.8	80.2	20.5	1	Insavc-1.pro
2	23.5	–	83.5	83.5	91.8	19.4	2	Tn449.pro
3	13.4	17.8	–	100.0	83.5	20.1	3	1-71.pro
4	13.4	17.8	0.0	–	83.5	20.1	4	Nvsl.pro
5	22.1	8.8	17.8	17.8	–	20.1	5	Ucd-2.pro
6	157.5	150.7	150.7	150.7	150.7	–	6	Ucd-1.pro
							–	

region to the FIPV 79–1146 S-gene. This contrasts with the other five CCV strains which were more similar to FIPV (78–87% identity) than to TGEV (25–27% identity).

3.3. Adaptation to ST cells

The two California CCV strains UCD-1 (TGEV-like) and UCD-2 (FIPV-like) were compared for growth on ST cells because this cell line is used for TGEV replication but does not readily support the growth of CCVs. After one blind pass on ST cells, fluorescent antibody positive cells were detected only for the CCV UCD-1 strain (Fig. 3). In addition, supernatant from the second pass of strains UCD-1 and UCD-2 was titrated on FC cells to measure any progeny virus. Whereas no progeny virus was detected for CCV strain UCD-2, the UCD-1 strain had a titer of 4.4×10^4 PFU/ml.

4. Discussion

Coronavirus strains isolated from dogs (CCVs), FCoVs and TGEV form an antigenic cluster group. Within this group, CCVs and FCoVs are similar at the very 3′ end of the genome in that they contain two ORFs, 7a and 7b, downstream of the nucleocapsid gene. In addition, the S gene of CCV strains Insavc-1, K378, CCV-6 and C54 have been sequenced and shown to be more similar to FIPV than to TGEV based on identities at the N-terminal domain of this gene (Horsburgh et al., 1992; Wesseling et al., 1994; Horsburgh and Brown, 1995). Similarly, in this paper, CCV strains NVSL, TN449, 1-71 and UCD-2 were also shown to be FIPV-like in terms of the N-terminal end of the S protein. However, one field strain, UCD-1, isolated in the late 1970s in California was more like TGEV in this region (amino acids 1–273) than like FIPV. Although the major coronavirus S gene epitopes are further downstream (residues 382–389; 538–543; 586–591), nevertheless, these differences could account partially for antigenic and host range diversity and may affect the efficacy of CCV vaccines.

Jacobs et al., (1987) was the first to show the divergence of S gene sequences between FIPV and TGEV and indicated that this region might be a potential cross-over site. These results support this idea in that most CCVs sequenced so far are FIPV-like at the N-terminus of the S protein but strain UCD-1 is TGEV-like. Thus, CCVs have sequences that are homologous to both feline and porcine CCVs. However, no FCoVs have been shown to have TGEV-like sequences in this N-terminal region nor have any swine coronaviruses been FIPV-like.

Moreover, CCV field strains may vary in their ability to infect different animal hosts and different cell culture systems. Woods and Wesley (1986, 1992) were able to infect pigs directly with CCV strain UCD-1 or by contact with dogs infected with an older NVSL challenge strain (DK-1). In contrast, pigs were not infected by CCV strain 1-71 (Binn et al., 1974). Woods and Wesley (1986) hypothesized that these differences could be due to the inoculating dose of virus or to cell culture adaptation of the UCD-1 strain. Another expla-
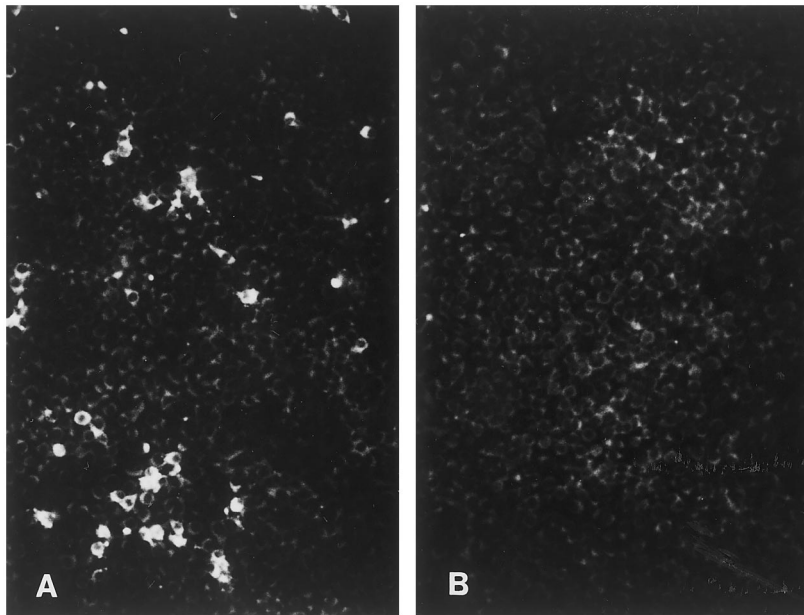
Fig. 3. Photomicrographs of fluorescent antibody positive swine testicular (ST) cells. Swine testicular cells were infected with (A) CCV UCD-1 or (B) CCV UCD-2. Supernatants were passaged once and after incubation for 4 days the cells were fixed and stained for CCV antigen. (Magnification 135 ×).

nation might be that CCVs vary in host range infectivity based on S gene sequence differences or other differences in the genome. The old NVSL challenge strain, DK-1, used by Woods and Wesley (1992) to infect dogs and then was transmitted to contact pigs is not the same as the current NVSL challenge strain (95-08). Sequencing of the S gene of the current NVSL challenge strain suggests that it might not be as infectious for pigs.

In terms of infectivity for ST cells, there is a clear difference between CCV strain UCD-1 which easily adapted to growth in ST cells and strain UCD-2 which did not. CCV strains 1–71, TN449 and NVSL are like UCD-2 and did not adapt easily. In contrast, the reference strain In-savc-1, readily infected ST cells on the first pass indicating that more than N-terminal S gene sequences are involved in ST cell infectivity. The reason for the broader infectivity of the Insavc-1 strain for cell cultures is not known. However, taken together, these results suggest that the CCV UCD-1 strain has an increased ability to infect porcine cell cultures, and perhaps, it is more

infectious for pigs. The TGEV-like sequences at amino acids 1–273 of the S gene may play a role that facilitates these properties.

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