JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1987, p. 1591–1596
0095-1137/87/091591-06$02.00/0

Vol. 25, No. 9

Diagnosis of Porcine and Bovine Enteric Coronavirus Infections Using Cloned cDNA Probes

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Received 24 February 1987/Accepted 22 May 1987

Molecular clones representing the first 2,000 bases from the 3′ end of the porcine transmissible gastroenteritis coronavirus genome and the first 2,160 bases from the 3′ end of the bovine enteric coronavirus genome were used in dot blot hybridization assays to detect viral RNA from cell culture and from fecal specimens. In each case, the cloned DNA represents approximately 10% of the genome. The cloned sequence for each virus encompasses the 3′ noncoding region, the nucleocapsid protein gene, and a large portion of the matrix protein gene. ^{32}P-labeled cDNA probes prepared from these clones detected as little as 25 pg of RNA from the parental virus but did not detect RNA from the nonparental virus even when amounts of up to 10 ng per dot were used. This specificity reflects the antigenic diversity between these two coronaviruses. The hybridization assay could also detect coronaviruses antigenically closely related to the parental virus but not coronaviruses belonging to an antigenically unrelated subgroup. Dot blot hybridization for transmissible gastroenteritis coronavirus diagnosis was compared with the routine procedures of virus isolation and electron microscopy as a diagnostic test.

Both porcine transmissible gastroenteritis coronavirus (TGEV) and bovine enteric coronavirus (BCV) cause significant economic loss through the death of young animals, and neither is effectively controlled by vaccination. Although they cause similar diseases in their respective hosts, they appear to be evolutionarily diverged coronaviruses, since they share no antigens. Each, therefore, is a member of one of two separate antigenic subgroups of mammalian coronaviruses (19). One major problem regarding coronavirus infections is the unavailability of a rapid and efficient means for identifying and quantifying viruses in infected animals. Such a test is important not only for differentiating coronaviruses from other agents that cause similar disease but also for determining the mechanism by which coronaviruses perpetuate enzootic or epizootic outbreaks. Presumably, persistently infected adult animals shed the virus and initiate outbreaks in susceptible animals, but this needs to be studied further. A sensitive test is also needed to determine the role of coronaviruses in acute enteric diseases of humans, putative zoonoses, and chronic diseases of animals and humans for which causes are not yet known (3, 4, 6, 25).

We have prepared cloned cDNA of specifically defined sequence from TGEV and BCV genomic RNA (10, 14). Clone FG5, which represents the first (3′) 2,000 bases of the 20,000-base TGEV genome, and clone MN3, which represents the first (3′) 2,160 bases of the 20,000-base BCV genome, were selected for use in this study. For both viruses, this region includes the complete 3′ noncoding region, the nucleocapsid (N) protein gene, and a large portion of the matrix (M) protein gene, as determined by an analysis of the primary structure of these sequences (10, 14; P. A. Kapke, F. Y. C. Tung, D. A. Brian, R. D. Woods, and R. Wesley, Adv. Exp. Med. Biol., in press). Since the N protein is completely intravirion and the M protein is largely so (23), these proteins would be the most likely among the viral proteins to escape immune pressures toward genetic variation. DNAs encoding these proteins would therefore be sequences of first choice for developing diagnostic hybridization probes that should recognize several strains (serotypes) of the same virus and perhaps recognize other viruses in the same antigenic subgroup, depending on the degree of nucleotide sequence conservation. On the basis of sequence analyses, we know that the nucleotide sequence homologies between the TGEV and BCV N and M protein genes are 37 and 51%, respectively (10, 14; Kapke et al., in press). Thus, probes prepared from these clones should be specific for their respective parental viruses and therefore useful in developing a diagnostic test.

In this study, we describe the use of cloned TGEV and BCV cDNA as hybridization probes that serve to identify and differentiate between these viruses and further demonstrate the usefulness of cloned TGEV cDNA for the diagnosis of TGEV infection in baby pigs.

MATERIALS AND METHODS

Cells and viruses. The Purdue strain of porcine TGEV, the Mebus strain of BCV, the OC43 strain of human respiratory coronavirus (HCV), the A59 strain of mouse hepatitis virus (MHV), the Indiana strain of vesicular stomatitis virus, and the Lasota strain of Newcastle disease virus were grown as previously described (5, 7, 13). Canine coronavirus and feline infectious peritonitis virus were grown on a fetal cat cell line (established by R. Woods), the 67N strain of porcine hemagglutinating encephalomyelitis virus (HEV) was grown on the human rectal tumor cell line HRT-18 (13), and the 229E strain of HCV was grown on WI-38 cells (American Type Culture Collection) using methods essentially identical to those previously described (7).

Preparation of virion RNA for nitrocellulose binding. Methods used for infection of cells, virus purification, and virion RNA extraction were described previously (5, 13). For these

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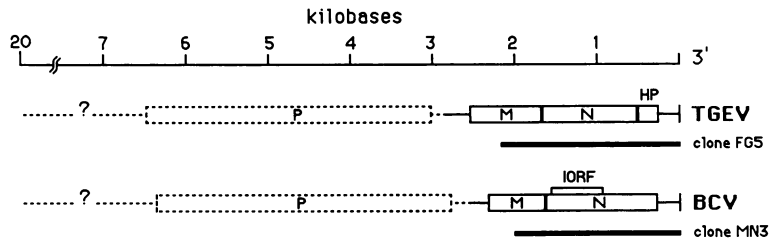

FIG. 1. Region of TGEV genome represented by clone FG5 (TGEV probe) and of BCV genome represented by clone MN3 (BCV probe). On top is a linear representation of the 20-kilobase coronavirus genome, with the 3' end positioned at the right. Regions representing the published sequences of the TGEV and BCV genomes encompassing the M protein, N protein, and HP (hypothetical hydrophobic protein) genes and IORF (internal open reading frame) are shown as boxes made with a solid line. The approximate region of the P (peplomeric protein) gene (made on the basis of partial sequence data) and the remainder of the genome are shown by broken lines. Regions represented by clones FG5 and MN3 are shown by heavy solid lines.

studies, virus (BCV, TGEV, or MHV) was purified from clarified supernatant fluids by being pelleted through a barrier of 32% sucrose (wt/wt) made up in TMEN (100 mM Tris maleate [pH 6.0], 100 mM NaCl, 1 mM EDTA). Virion RNA was extracted using the proteinase K-sodium dodecyl sulfate-phenol method (5). RNA was dissolved in water and spectrophotometrically quantitated assuming that 1 A_{254} unit in 1 ml is equal to 42 μg of RNA. RNA was handled at all times in baked or autoclaved siliconized containers and prepared in solutions made from diethylpyrocarbonate-treated water.

For nitrocellulose binding, purified virion RNA, cell culture-grown virus, or fecal specimens were treated essentially as described by White and Bancroft (26) for the binding of cytoplasmic RNA. Preliminary experiments demonstrated that Nonidet P-40 (NP-40) (Bethesda Research Laboratories, Inc.) enhanced the binding of RNA to nitrocellulose, even in its purified form. RNA was therefore dissolved and diluted in TE buffer (10 mM Tris [pH 7.0], 1 mM EDTA), and each sample was made 0.5% NP-40 by adding an equal volume of 1% NP-40 in TE buffer. To each NP-40-treated sample was added an equal volume of a freshly prepared solution of two parts of 37% (wt/wt) formaldehyde and three parts of 20× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) (pH 7.0), and the mixture was heated at 60°C for 15 min just before being loaded onto nitrocellulose. RNA dilutions were made in siliconized 96-well microtiter plates.

Preparation of cell culture-grown virus for nitrocellulose binding. Cells were infected at a multiplicity of approximately 1 PFU per cell, and cell culture fluids were harvested at 17, 48, and 10 h postinfection, respectively, for TGEV, BCV, and MHV. Culture fluids were clarified by centrifugation at 2,000 × *g* for 10 min and stored at −80°C until use. Virus dilutions were made in TE buffer, and virus was lysed by the addition of an equal volume of 1% NP-40 in TE buffer. Samples were incubated for 10 min at 0°C, treated with an equal volume of formaldehyde–20× SSC solution, and heated at 60°C for 15 min before being loaded onto nitrocellulose.

Preparation of fecal specimens for nitrocellulose binding. Individuals in a litter of 12 3-day-old pigs were given 5 ml of inoculum of a given dilution of TGEV stock by stomach tube. Virus stock had a titer of 7.1×10^6 PFU/ml and was prepared from small-intestinal contents of a piglet that had been infected with the TGEV Miller strain. Virus used for inoculating the piglet from which virus stock was prepared had undergone 11 passages in gnotobiotic pigs after plaque purification in cell cultures (8). Pigs 1 and 2 were inoculated with a 10^{-1} dilution, pigs 3 and 4 were inoculated with a 10^{-2} dilution, pigs 5 and 6 were inoculated with a 10^{-3} dilution, pigs 7 and 8 were inoculated with a 10^{-4} dilution, pigs 9 and 10 were inoculated with a 10^{-5} dilution, and pigs 11 and 12 were inoculated with a 10^{-6} dilution of virus stock. Fecal specimens were taken with dry cotton swabs and stored at −80°C until use. To prepare samples, 0.75 ml of sterile Earle balanced salt solution was added to the tube containing the swab and, after being vortexed briefly, the fluid was removed and clarified by Microfuge centrifugation for 30 s. Clarified fluid was treated for nitrocellulose binding either directly (termed undiluted) or after being diluted 1:10 in TE buffer. Virus was lysed and treated for nitrocellulose binding as described above for cell culture-grown virus.

Binding of RNA-containing samples to nitrocellulose. Nitrocellulose (BA85; Schleicher & Schuell, Inc.) was soaked in diethylpyrocarbonate-treated water and equilibrated with 20× SSC before application of samples. Nitrocellulose was supported by two sheets of blotting paper in a 96-hole dot blot apparatus (Minifold; Schleicher & Schuell, Inc.). A 100-μl sample prepared as described above was applied to each well. Samples of purified RNA were allowed to set for 30 s before light suction was applied. With all other samples, strong suction was applied immediately. Treated nitrocellulose sheets were air dried and baked at 76°C for 90 min in a vacuum oven to fix the RNA (24).

Preparation of cloned cDNA probes and molecular hybridization. cDNA clones of TGEV and BCV representing the 3' ends of their genomes have been prepared and characterized (Fig. 1; 10, 14). cDNA was cloned into plasmid pUC9 using *Escherichia coli* JM103 as the host. The insert-containing plasmid was obtained from cultured bacteria using lysozyme and alkali and purified on CsCl gradients (16). Insert DNA was cleaved from the plasmid using restriction endonucleases *Bam*HI and *Hin*dIII (Pharmacia, Inc.). BCV clone MN3 yielded the insert as a single 2.16-kilobase fragment, whereas the TGEV clone FG5 yielded two fragments of 1.3 and 0.7 kilobases because of an internal *Hin*dIII cutting site. Fragments were recovered by preparative electrophoresis in 1% agarose gels and electroelution (16). Purified inserts were labeled to specific activities of 1×10^8 to 4×10^8 cpm/μg by nick translation with ^{32}P (22). Before they were used for hybridization, probes were denatured by heating for 5 min at 100°C, followed by quick cooling on ice.

Hybridization was done essentially as described by Thomas (24), but without dextran sulfate. Blots were incubated (prehybridized) for 4 h at 42°C in sealed plastic bags containing a solution (1 ml/cm^2) of 50% formamide–5× SSC–50 mM Na_2HPO_4 (pH 6.5)–0.2% sodium dodecyl sulfate–1× Denhardt solution (0.02% bovine serum albumin,

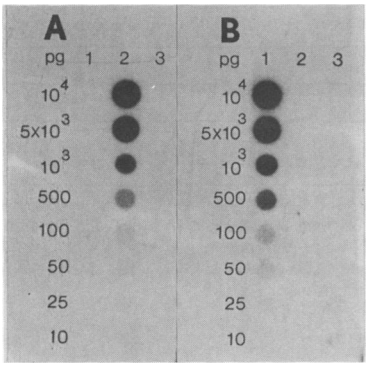

FIG. 2. Sensitivity and specificity of cloned TGEV and BCV cDNA probes for different RNAs. (A) TGEV probe. (B) BCV probe. Columns: 1, dilutions of BCV RNA; 2, dilutions of TGEV RNA; 3, dilutions of yeast transfer RNA. Blots were autoradiographed at −70°C for 48 h with an intensifying screen.

0.02% polyvinylpyrrolidone, 0.02% Ficoll [Pharmacia])–250 μg of sheared, denatured salmon sperm DNA per ml. Fluids were removed, and fresh solution (1 ml/cm^2) and denatured cDNA probe (10^6 cpm/cm^2) were added to the bag. The bag was sealed and incubated at 42°C for 16 to 24 h, and the nitrocellulose was washed four times with 2× SSC–0.1% sodium dodecyl sulfate for 5 min at room temperature and then twice with 0.1× SSC–0.1% sodium dodecyl sulfate for 15 min at 50°C. Dried blots were exposed to Kodak AR X-ray film at −70°C with an intensifying screen for 24 to 48 h.

Immunofluorescence testing. Immunofluorescence testing was performed essentially as described by Potgieter and Aldridge (20). Cells were scraped from the flask, washed, suspended in phosphate-buffered saline, spread onto glass slides, dried, fixed with acetone, incubated with TGEV-positive (hyperimmune) pig serum or preimmune serum, and stained with fluorescence-labeled rabbit anti-swine immunoglobulin G (Miles Laboratories, Inc.).

Electron microscopy. Fecal samples were prepared for electron microscopy essentially as described by Almeida (1). A portion of the clarified resuspended fecal material was centrifuged at 15,000 × *g* for 10 min, and the pellet was suspended in a minimal amount of deionized water. One volume of suspended pellet was mixed with nine volumes of deionized water and one volume of 4.0% phosphotungstic acid (neutralized to pH 7.0) and sprayed onto a Formvar-carbon-coated grid using a glass nebulizer.

RESULTS

Specificity and sensitivity of cloned cDNA probes in dot blot hybridization reactions. To determine the specificity and sensitivity of each probe for its respective viral RNA, RNA extracted from purified TGEV, BCV, or yeast transfer RNA was applied to nitrocellulose in amounts ranging from 10 pg to 10 ng per dot and tested for hybridization with the TGEV or BCV probe. Each probe bound specifically to its homologous RNA, and no reactivity with heterologous RNA was observed (Fig. 2). Furthermore, each probe was able to detect as little as 25 pg of homologous RNA per dot, the amount of RNA from 2 × 10^6 virions, assuming a genome size of 7 × 10^6 daltons for both TGEV and BCV (5, 13). Dot blots of 10 ng of MHV, Newcastle disease virus, and vesicular stomatitis virus RNA were also tested with each probe, and no detectable binding was observed (data not shown). These results were surprising since we expected the BCV probe to bind to RNA from the antigenically related MHV. Detailed analyses revealed, however, that because of different codon usage the genes of the N and M proteins of BCV and MHV share a low degree of nucleotide sequence homology despite a high degree of amino acid sequence conservation in the proteins (14). The BCV probe did bind to MHV RNA when more than 10 ng per dot was used and washes were of the stringency described above (data not shown).

Detection of RNA by dot blot hybridization in virus prepared directly from cell culture fluids. Cell culture fluids from virus-infected cells were used first to establish a procedure for detecting virus from body fluids and second to examine the usefulness of the TGEV and BCV probes for identifying other coronaviruses. For the first purpose, a series of 10-fold dilutions of clarified cell culture fluids from TGEV-, BCV-, and MHV-infected cells were denatured and applied to nitrocellulose. The probes hybridized in a specific manner to parental RNAs, except for the lowest virus dilutions, at which some TGEV probe bound to BCV but not to MHV and some BCV probe bound to MHV but not to TGEV (Fig. 3). The apparent hybridization at low dilutions of virus in the first case was probably artifactual, since there was no reciprocal hybridization between the BCV probe and TGEV. In the second case, hybridization of the BCV probe with MHV apparently reflected an MHV RNA concentration of more than 10 ng per dot. In other blotting experiments, both probes failed to hybridize to vesicular stomatitis virus from cell culture fluids, and this further established the specificity of the probes (data not shown). An interesting observation is that for both TGEV and BCV, dilutions of up to 10^{-3} yielded a positive hybridization signal, suggesting that as much as 25 pg of RNA was present in the 10^{-3} dilution. Since at this dilution the amount of sample deposited is equivalent to a volume of 0.01 μl of undiluted cell culture fluid, the number of virus particles per milliliter is calculated to be approximately 2 × 10^{11} (assuming there is 1 genome of 7 × 10^6 daltons per virion and that 25 pg of RNA is equivalent to 2 × 10^6 virions). This is 100- to 400-fold greater than the highest infectivity titers we have observed for cell culture-grown TGEV and BCV as measured in PFU per milliliter (5, 11) and suggests that there is a ratio of noninfectious-to-infectious virions ranging from 100 to 400 in the cell culture fluids.

To examine the reactivity of TGEV and BCV probes with other coronaviruses, various cell culture-grown coronaviruses were applied to nitrocellulose in the same manner,

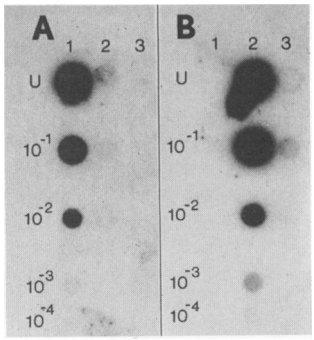

FIG. 3. Detection of viral RNA directly from cell culture fluids. (A) TGEV probe. (B) BCV probe. Columns: 1, dilutions of TGEV; 2, dilutions of BCV; 3, dilutions of MHV. Blots were autoradiographed at −70°C for 48 h with an intensifying screen.

TABLE 1. Dot blot hybridization using cloned TGEV and BCV probes on various coronaviruses obtained from cell culture fluids

Probe[a]	Hybridization with[b]:							
	TGEV	FIPV	CCV	HCV 229E	BCV	HEV	HCV OC43	MHV A59
TGEV	++++	++++	++++	−	−	−	−	−
BCV	−	−	−	−	++++	++++	+++	+

[a] Purified cloned insert DNA for TGEV or BCV was radiolabeled by nick translation and used in hybridization tests as described in Materials and Methods.
[b] Supernatant fluids from infected cell cultures were denatured and applied to nitrocellulose as described in Materials and Methods for an undiluted sample. −, No hybridization observed; +, dot density was equivalent to what was observed when 25 to 1,000 pg of homologous RNA per dot was used in the standard; +++, density when 10 to 50 ng per dot was used; ++++, density when >50 ng per dot was used. Abbreviations: BCV, BCV Mebus strain; CCV, canine coronavirus; FIPV, feline infectious peritonitis virus; HEV, HEV strain 67N; TGEV, TGEV Purdue strain.

except only for a limited number of dilutions. The results for undiluted samples are shown in Table 1. In general, probes did not react with viruses belonging to the unrelated antigenic subgroup (19), nor did they identify all the viruses antigenically related to the parent of the clone. The BCV probe hybridized with HCV OC43 and HEV but not with MHV, and the TGEV probe hybridized with canine coronavirus and feline infectious peritonitis virus but not with HCV 229E. These differences undoubtedly reflect evolutionary distances between the viruses and illustrate the potential usefulness of hybridization probes for the eventual identification of coronavirus subgroups.

Detection of TGEV RNA in virus from fecal samples and comparison of dot blot hybridization with other diagnostic methods. To establish the usefulness of the dot blot procedure for identifying coronaviruses in fecal samples, a litter of 12 piglets was experimentally infected with TGEV as described in Materials and Methods, and fecal swabs were taken at 0, 18, 30, 44, 90, and 114 h postinfection from each survivor. Swabs were processed for dot blot hybridization against the TGEV probe (Fig. 4), and swabs from six of the animals were additionally processed for diagnosis by electron microscopy and infectivity (Table 2).

The following points emerge from these studies. (i) The abundance of TGEV particles in the feces of infected animals is more than adequate for detection by the dot blot hybridization test. Since the amount of feces absorbed by the cotton swab is approximately 0.5 ml, the volume of fecal equivalents adsorbed in one dot of an undiluted sample is approximately 10 μl. A dot of fecal sample containing 25 pg of RNA would therefore be equivalent to 2×10^8 virions per ml of feces if the sample is undiluted or 2×10^9 virions per ml if the sample is diluted 1:10. Dots having 50 μg of virion

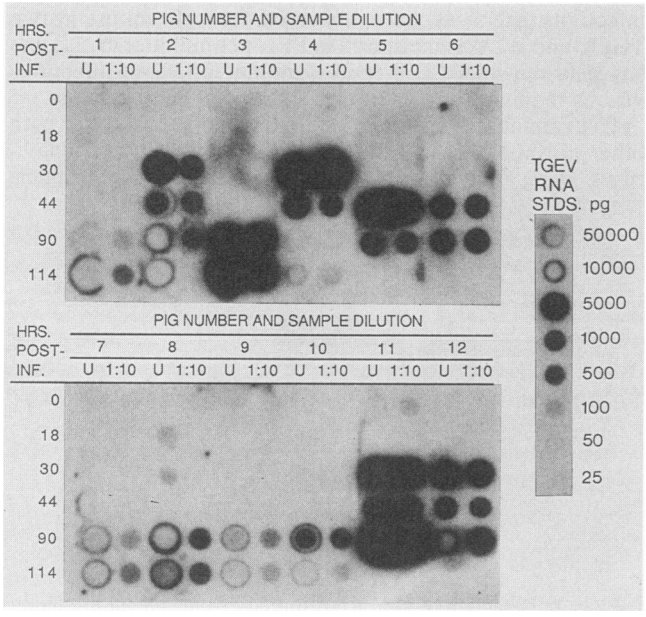

FIG. 4. Dot blot hybridization detection of TGEV RNA in fecal specimens of experimentally infected piglets. Blots were autoradiographed at −70°C for 48 h with an intensifying screen.

TABLE 2. Comparison of dot blot hybridization with other methods of detecting TGEV in fecal specimens

Pig no.	Inoculum (PFU/pig)	Time post-infection (h)	Virus detection by:		
			EM[a]	Isolation[b]	Dot blot hybridization[c]
3	35.5×10^4	0	−	−	−
		18	+	−	−
		30	ND	−	−
		44	+	−	−
		90	+	+	++++
		114	+	+	++++
4	35.5×10^4	0	−	−	−
		18	+	−	−
		30	+	+	++++
		44	−	+	++
		90	−	+	+
		114	ND	+	+
5	35.5×10^3	0	−	−	−
		18	+	−	−
		30	−	−	−
		44	+	+	+++
		90	+	−	++
6	35.5×10^3	0	−	−	−
		18	ND	−	−
		30	+	−	−
		44	ND	+	+
		90	ND	+	+
11	35.5	0	−	−	−
		18	ND	−	−
		30	+	+	++++
		44	−	+	++
		90	+	+	++++
12	35.5	0	−	−	−
		18	−	−	−
		30	+	+	++
		44	+	+	+
		90	+	+	++

[a] Fecal material was prepared for electron microscopy (EM) as described in Materials and Methods. +, One or more coronavirus particles observed in a field of six droplets; −, no coronavirus particles observed; ND, not done.
[b] Swine testicle cells (17) were inoculated with fecal specimens and evaluated for cytopathic effect after 3 days. Cells were then scraped from flasks and prepared for immunofluorescence as described in Materials and Methods. +, Immunofluorescence; −, no immunofluorescence.
[c] Estimated viral RNA concentrations: −, <25 pg per dot; +, 5 to 1,000 pg per dot; ++, 1 to 10 ng per dot; +++, 10 to 50 ng per dot; ++++, >50 ng per dot.

RNA or more, as observed at peak times for animals 3, 4, 5, 11, and 12, would therefore be equivalent to fecal concentrations of at least 4×10^{11} and 4×10^{12} virions per ml for samples that are undiluted or diluted 1:10, respectively. (ii) The time of virus appearance in feces did not reflect the inoculum size. Animals receiving 35 PFU (numbers 11 and 12) began shedding virus earlier than animals receiving a far larger inoculum (e.g., animal 1 that received 35×10^5 PFU and animal 3 that received 35×10^4 PFU). In no animal was viral RNA detectable until more than 18 h after infection. Four piglets (numbers 5, 6, 11, and 12) did not survive to 114 h postinfection and, for these, the 90-h sample was the last one taken. (iii) Abundance patterns in two of the animals (numbers 2 and 4) suggest that there may be both a rapid onset and a rapid decline in the number of excreted viruses during the 3-day course of infection, although a wide variation in sampling quantities that could also explain this pattern cannot be ruled out. For these animals, virus appeared to be most abundant at 30 h postinfection and quantities decreased from 44 through 114 h postinfection. (iv) The high viscosity of some undiluted samples interfered with RNA adherence to the nitrocellulose. This can be seen for piglets 1, 2, 4, 7, 8, and 12 at the 90-h timepoint and for piglets 1, 2, 4, 7, 9, and 10 at the 114-h timepoint, for which samples appeared to peel off the nitrocellulose during processing. Since these experiments were done, we have learned that a bubble often forms under a viscous sample as it is applied. This could have caused the poor adhesion observed for some fecal samples and also for the RNA standards of 10,000 and 50,000 pg. We now know that removing the bubble before suction is applied allows for a rapid sample flow rate and an even distribution of RNA on the filter.

When comparison was made between the dot blot hybridization test and other methods of diagnosis on six of the animals (Table 2), the best correlation was found with the virus isolation test as confirmed by immunofluorescence. In only one case, animal 5 at 90 h postinfection, did the virus isolation and hybridization tests disagree. In this case, the sample was taken during the declining stages of infection, a time when the number of viable viruses may be low relative to the total number, thus yielding a positive test by hybridization and a negative test by infectivity. Less correlation was observed between the dot blot hybridization test and electron microscopy. Generally, virus was detected sooner by electron microscopy than by virus isolation and dot blot hybridization; however, the particles observed may have been only coronaviruslike particles of the type commonly observed in the diseased gut and not true coronaviruses (2).

DISCUSSION

The special usefulness of nucleic acid hybridization for rapid, specific, and sensitive detection of viruses in both body fluids and tissues was recently reviewed (21). Before this method or any of its modifications can be applied to detect coronaviruses, viral cDNA of defined sequence must be molecularly cloned and characterized. We report here the successful use of cDNA clones prepared from the genomic RNA of two medically important but antigenically unrelated enteric coronaviruses, TGEV and BCV (10, 14), for the specific detection of RNA from their respective parental viruses. Two features of this study demonstrate the feasibility of developing the nucleic acid hybridization test for the clinical diagnosis of enteric coronavirus infections. First, the sensitivity of 25 pg per dot is 400-fold greater than that of a similar test developed for detection of rotaviruses in clinical specimens (15). The greater sensitivity in the coronavirus test may be a function of the single-stranded genome, since a complementary RNA strand does not interfere in the assay. Second, TGEV clones were useful for detection of TGEV in the feces of infected pigs, with a sensitivity equal to that of a virus isolation test routinely used for TGEV diagnosis. This is especially important in light of the fact that hybridization tests have not been universally successful for detecting single-stranded RNA enteroviruses in feces (9). Improved utility of the coronavirus hybridization test could be developed by using a nonradioactive label, such as biotin (12), and sensitivity could possibly be enhanced by the use of single-stranded RNA probes prepared from the cloned sequences (18).

Preliminary experiments also demonstrated that the cloned cDNA is useful in identifying many but not all coronaviruses classified in the same antigenic subgroup as parental viruses. That is, cDNA to TGEV identified feline infectious peritonitis virus and canine coronavirus but not HCV 229E, and cDNA to BCV identified HEV and HCV OC43 but not MHV. The exact regions of genomic sequence giving rise to hybridization will of course become known as primary sequence data become available for individual coronaviruses. The specificity of any given probe could theoretically be chosen by selecting sequences of defined uniqueness for any virus or group of viruses. For example, the N gene for BCV, having a nucleotide sequence homology of 71% with the analogous gene of MHV, would allow for greater discrimination between these two viruses than would the M gene alone, with a sequence homology of 79% (14).

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant AI-14367 from the National Institute of Allergy and Infectious Diseases, by grant 82-CRSR-2-1090 from the U.S. Department of Agriculture, and in part by a grant from the National Foundation for Ileitis and Colitis, Inc.

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