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Detection of Infectious Bursal Disease Viruses by Using Cloned cDNA Probes†

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A molecular clone representing 445 base pairs at the 3′ end of infectious bursal disease virus (IBDV) genome segment B was used in a dot blot hybridization assay to detect viral RNA from cell culture and from chicken bursa and spleen tissue specimens. The cloned nucleotide sequence represents approximately 14% of the virus-encoded polymerase (VP-1) gene. The lower detection limit of radiolabeled probes prepared from this clone was 0.1 ng of IBDV double-stranded RNA. The probe had broad specificity and was used to detect four serotype 1 IBDV strains and one serotype 2 IBDV strain. This probe, however, did not cross-react with nucleic acid extracted from nine unrelated poultry viruses. A rapid procedure for isolation of IBDV genomic RNA from bursa and spleen tissue specimens was developed and used with the dot blot hybridization assay to detect IBDV strains in tissue samples from experimentally infected and commercially reared chickens.

Infectious bursal disease virus (IBDV) infections have been observed in chickens since 1957 (5). It causes an immunosuppressive disease in young chickens. Initially only one serotype of this birnavirus was thought to exist. However, a second serotype isolated from turkeys was designated serotype 2, and previously known isolates of IBDV from chickens were designated serotype 1 (13, 18). No pathogenic serotype 2 viruses have been isolated, and serotype 1 viruses are pathogenic only in chickens. Several antigenic subtypes of serotype 1 viruses have been described (12).

The IBDV genome consists of two segments of double-stranded RNA (dsRNA). The largest segment (A) is approximately 3,400 base pairs long and contains a large open reading frame which is translated into a polyprotein (11). The smaller segment (B) is approximately 2,900 base pairs long and encodes a 90,000-molecular-weight protein (20). Differences in the electrophoretic mobilities of the genome segments have been observed between serotype 1 and 2 viruses (3, 14). The nucleotide sequence homology of genome segments from different IBDV isolates has not been reported.

Diagnostic assays currently used for IBDV infection are the agar-gel precipitin test, the enzyme-linked immunosorbent assay, the virus neutralization assay in cell culture and embryonated chicken eggs, immunofluorescence techniques, and electron microscopy. The enzyme-linked immunosorbent assay is used to detect antibodies to the virus. It is fast and sensitive but may not be a reliable diagnostic assay for IBDV early in infection (7). Neutralization assays and the agar-gel precipitin test are also designed to detect antibodies to IBDV. Compared with the enzyme-linked immunosorbent assay, neutralization assays are more difficult and time consuming and the agar-gel precipitin test is less sensitive. Immunofluorescence techniques are used to detect viral antigens in tissue sections or impression smears (1, 16). They are not used routinely for IBDV because it is difficult to test large numbers of samples, expensive equipment is required, and false-negative results have been obtained with impression smear samples (1). A diagnostic assay which is not only sensitive and fast but can be adapted to test many samples for IBDV infections is needed.

Improved methods of DNA cloning and hybridization have led to the development of diagnostic assays which use nucleic acid probes to detect human and animal pathogens (25). To examine the possibility of using DNA probes to detect IBDV, molecular clones of the virus were prepared in our laboratory. In this communication, we describe the preparation of DNA probes from a cloned segment of the IBDV genome and the use of these probes to detect viral dsRNA in cell culture samples and tissue specimens from infected birds. Our results demonstrate the usefulness of IBDV cDNA probes for the rapid diagnosis of IBDV infections in poultry.

MATERIALS AND METHODS

Viruses. The BB isolate is an attenuated vaccine strain of IBDV which was commercially available (Bio-Burs; Agri Bio Corp., Ithaca, N.Y.). The genome of this virus was used to obtain the cDNA clones used for production of the probes. Six IBDV isolates, representing both serotypes (1 and 2) and four antigenic subtypes of serotype 1, were used in the dot-blot hybridization assays. The serotype and subtype of the viruses used in this study were previously described (12) and are presented in Table 1. Three of these isolates are commercially available serotype 1 vaccine strains: SAL (Bursine; Salsbury Laboratories, Charles City, Iowa), D-78 (Clonevac-D78; Intervet America Inc., Millsboro, Del.), and UV (American Scientific Laboratories, Omaha, Nebr.). The OH, MD, and E viruses are field isolates. The OH virus was isolated from turkeys in Ohio (13). It is a serotype 2 virus which is nonpathogenic in chickens or turkeys. The MD and E viruses are relatively new serotype 1 isolates from chickens and usually referred to as variants (12). The highly pathogenic standard challenge virus (ST-C) is used by the U.S. Department of Agriculture to evaluate the efficacy of serotype 1 vaccines. This serotype 1 virus has not been adapted to cell culture and thus was

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TABLE 1. Serotype and subtype specificities of IBDV isolates

Virus isolate	Serotype	Subtype[a]
BB	1	A
SAL	1	A
UV	1	B
D-78	1	C
MD	1	F
E	1	F
ST-C	1	ND[b]
OH	2	NA[c]

[a] Six antigenic subtypes of serotype 1 viruses were identified (11) and designated A to F.

[b] ND, Not determined. The highly pathogenic ST-C virus was not placed into an antigenic subtype because it is not adapted to replicate in cell culture.

[c] NA, Not applicable. The OH and other serotype 2 viruses were not classified as to antigenic subtype because of the limited number of viruses which have been isolated.

propagated in 3-week-old specific-pathogen-free chicks (13). Because it is not adapted to cell culture, its antigenic subgroup has not been determined. All viruses used in these studies except ST-C were grown and their titers were determined in grivet monkey kidney cells (BGM-70; 15).

Nine poultry viruses were used to determine the specificity of the BB-15 probe. The viruses tested were obtained from a commercial vaccine manufacturer (American Scientific Laboratories) and included infectious laryngotracheitis virus (herpesvirus), fowl poxvirus, pigeon poxvirus, infectious bronchitis virus (coronavirus), LaSota and B1 Newcastle disease viruses (paramyxoviruses), hemorrhagic enteritis virus (adenovirus), turkey herpesvirus (Marek's disease vaccine), and an avian reovirus.

RNA extraction and purification. Virus particles were harvested from cell culture fluid by using Freon (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) extraction as previously described (14). Chicken bursas containing the ST-C virus were homogenized and then frozen and thawed three times before Freon extraction. Freon-extracted viruses were concentrated by centrifugation at 132,000 × *g* for 3 h at 4°C. Viral pellets were suspended in TNE (10 mM Tris [pH 8.0], 100 mM NaCl, 1 mM EDTA) containing 0.5% sodium dodecyl sulfate (SDS). Proteinase K (Sigma Chemical Co., St. Louis, Mo.) was added to a final concentration of 2.0 mg/ml, and the samples were incubated at 37°C for 2 h. The RNAs were extracted with phenol and chloroform and then precipitated in ethanol. Cellular DNA was removed by using RNase-free DNase I (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) at a concentration of 100 U/mg of total nucleic acid.

dsRNA was separated from single-stranded RNA by using a modification of the CF-11 cellulose column procedure of Mellor et al. (19). A 3-ml syringe was plugged with silicone-treated glass wool and filled with 1 g of CF-11 cellulose powder (Whatman International Ltd., Maidstone, England). Viral RNA samples were placed in STE (50 mM Tris [pH 7.0], 100 mM NaCl, 1 mM EDTA) containing 15% (vol/vol) ethanol. A 5.0-ml volume of STE-ethanol buffer was added to each column, followed by addition of the samples. Each column was washed with 10 ml of STE-ethanol buffer, and the dsRNA was eluted in 4.0 ml of STE without ethanol. Before cloning and hybridization procedures, the viral RNA was concentrated by ethanol precipitation.

Preparation of the BB-15 cDNA clone. The dsRNA genome segments of the BB virus were used. The IBDV RNA was placed in a 10-μl volume of 90% dimethyl sulfoxide and heated at 65°C for 90 min. Just before being added to the first-strand cDNA reaction mixture, the RNA was heated for an additional 3 min at 100°C. Random calf thymus DNA was used to prime the first-strand reaction. Denatured viral RNA (5.0 μg) and random primers (1.0 μg) were added to the reaction mixture containing 50 mM Tris (pH 8.0), 75 mM KCl, 10 mM $MgCl_2$, 10 mM dithiothreitol, and the deoxynucleoside triphosphates at 1.5 mM each. Reverse transcriptase (75 U; Boehringer Mannheim Biochemicals) was added, and incubation was at 42°C for 2 h. Following incubation, the samples were ethanol precipitated. ds cDNA was synthesized by the procedures of Gubler and Hoffman (9). The reagents in this reaction mixture were identical to those used in the first-strand reaction, except that DNA polymerase I (25 U; Boehringer Mannheim Biochemicals) was used in place of reverse transcriptase and 0.85 U of RNase H (Boehringer Mannheim Biochemicals) was added. Incubation was at 16°C for 2 h. Double-stranded cDNA was tailed by using dCTP and terminal transferase (Boehringer Mannheim Biochemicals) and then annealed into pUC9 previously restricted with *Pst*I and oligo(dG) tailed. The recombinant plasmids were used to transform *Escherichia coli* JM107 (27).

Virus-specific clones were identified by using a colony blot hybridization procedure (8). The BB-15 clone was selected from the virus-specific cDNA clones identified. Its location on the viral genome was determined by using Northern (RNA) blot hybridization and nucleotide sequence analysis (22, 24).

Northern blot hybridization. Purified dsRNA from IBDV strain BB was denatured and separated on a 1% agarose gel containing 2.2 M formaldehyde as previously described (17). Separated RNA segments were transferred to a nylon membrane (Biotrans; ICN Pharmaceuticals Inc., Irvine, Calif.) by using the capillary blot procedure (26), and the membrane was baked at 80°C for 1 h before hybridization.

Preparation of probes. Radiolabeled cDNA probes were prepared from the BB-15 clone. Plasmids containing the BB-15 insert were extracted as described previously (4). Cloned IBDV sequences in BB-15 were excised from pUC9 plasmids by using *Pst*I and separated on 6% polyacrylamide gels in TBE buffer (90 mM Tris, 90 mM boric acid, 1 mM EDTA [pH 8.3]). The cloned fragments were electroeluted from gels into TBE buffer before use in the nick translation reactions. Nick translations were conducted with 50 μCi of [^{32}P]dCTP (specific activity, >600 Ci/mmol; ICN Pharmaceuticals) (21). The probes were purified by centrifugation through Sephadex G-50 columns before use (17).

For colony blot and Northern blot hybridizations, probes were prepared directly from the purified viral dsRNA genome by using methods previously described (10; D. J. Jackwood, submitted for publication).

Preparation of IBDV RNA from infected cell cultures. Cell culture-grown virus was frozen and thawed three times. Viral RNA was prepared for hybridizations by using a modification of the procedure described by Shockley et al. (23). A 1.0-ml volume of cell culture fluid containing 0.5% Nonidet P-40 (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) was kept at 0°C for 30 min. The samples were then centrifuged at 15,000 × *g* for 2.0 min in a microcentrifuge, and 0.3 ml of supernatant was added to an equal volume of a freshly prepared solution containing 2 parts of 37% (wt/wt) formaldehyde and 3 parts of 20× SSC (3.0 M NaCl, 0.3 M sodium citrate; 1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). This solution was incubated at 60°C for 30 min. Following incubation, an equal volume of form-

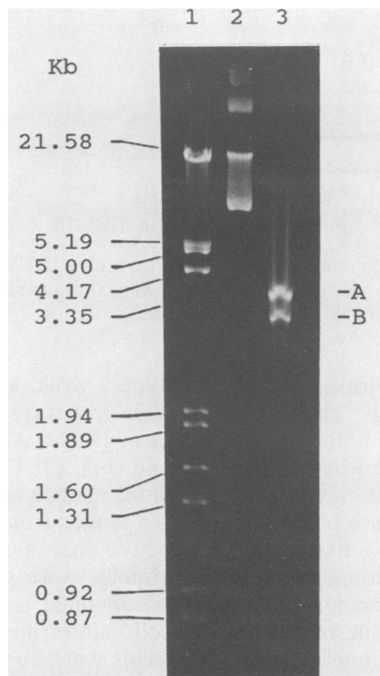

FIG. 1. Agarose gel electrophoresis of IBDV genomic RNA following purification with CF-11 cellulose. The nucleic acids were visualized after being stained with ethidium bromide. Lambda bacteriophage DNA digested with *Hin*dIII and *Eco*RI (lane 1) and pBR322 (lane 2) were included for size references. Viral genome segments A and B purified from IBDV strain BB (lane 3) are indicated. Kb, Kilobases.

amide (0.6 ml) was added and the dsRNA was denatured at 100°C for 3 min.

Preparation of IBDV RNA from tissue specimens. Bursa and spleen tissues were harvested from 4-week-old chicks at 2 or 3 days following intranasal inoculation with the ST-C virus. Bursa tissues were also obtained from chickens on five poultry farms with suspected cases of infectious bursal disease. Tissue samples were homogenized in TNE buffer, frozen and thawed three times, and then treated with proteinase K (2 mg/ml) in 0.5% SDS at 37°C for 2 h. Following incubation, the samples were extracted with phenol and chloroform. An equal volume of formamide was added, and the viral dsRNA was denatured at 100°C for 3 min.

Preparation of nucleic acid from poultry viruses. The specificity of the BB-15 probe for IBDV was determined by using the UV strain of IBDV and the nine unrelated poultry viruses listed above. The nucleic acid was extracted directly from virions contained in vaccine vials without further passage in cell culture. Each vaccine vial contained a minimum of 1,000 doses of infectious virus. The lyophilized viruses were suspended in TNE containing 0.5% SDS and treated with proteinase K (2.0 mg/ml), and the nucleic acids were extracted as described previously for bursa and spleen tissues. Alternatively, the lyophilized viruses were suspended in TNE and the nucleic acids were extracted as described previously for cell culture-propagated viruses.

Binding of viral nucleic acid to nylon membranes. IBDV RNA extracted from cell culture or tissue specimens and viral nucleic acids from vaccine viruses were placed on nylon membranes (Biotrans; ICN Pharmaceuticals) by using a 96-well filtration manifold (Hybri-dot; Bethesda Research Laboratories). The nylon membranes were hydrated in H_2O, followed by 20× SSC, and the samples were applied by using strong suction. Membranes containing viral RNA were air dried for 30 min and then baked at 80°C for 1 h.

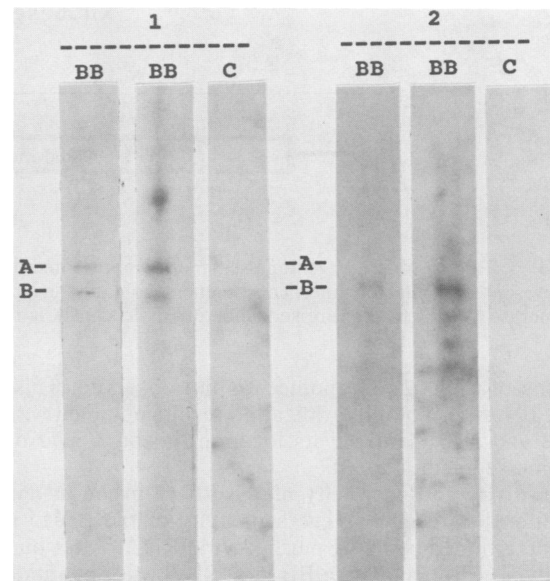

FIG. 2. Northern blot hybridization. IBDV genomic RNA (BB) and BGM-70 cell culture nucleic acid (C) were denatured, separated on a 1% agarose-formaldehyde gel, and blotted onto nylon membranes. Duplicate membranes were prepared. One of these membranes was incubated with a probe prepared from the BB genome (panel 1). The other filter was incubated with a probe prepared from the BB-15 clone (panel 2). Viral genome segments A and B are labeled.

Dot blot hybridizations. Nylon membranes containing viral nucleic acid samples were prehybridized for 4 h at 42°C in a hybridization solution containing 5× SSC, 5× Denhardt solution (0.1% [wt/vol] Ficoll, 0.1% [wt/vol] polyvinylpyrrolidone, 0.1% [wt/vol] bovine serum albumin), 50 mM sodium phosphate (pH 6.5), 0.1% (wt/vol) SDS, 250 μg of salmon sperm DNA per ml, and 50% (vol/vol) formamide. Following prehybridization, fresh hybridization solution and radiolabeled probes (3×10^6 cpm per membrane) were added. Incubation was continued at 42°C for 18 h. Hybridized membranes were washed at room temperature in 2× SSC containing 0.1% (wt/vol) SDS and then in 0.1× SSC containing 0.1% (wt/vol) SDS before autoradiography.

RESULTS

Characterization of the BB-15 cDNA clone. The viral RNA appeared to be extremely pure following CF-11 cellulose purification (Fig. 1). With colony blot hybridizations, IBDV-specific clones were identified in the BB cDNA library. Clone BB-15 was selected at random from the IBDV clones observed. Northern blot hybridization results indicated that clone BB-15 hybridized to viral genome segment B (Fig. 2). The cDNA probe prepared directly from the BB dsRNA genome was used as a control in the Northern blot hybridization. This probe hybridized to both segments of the genome (Fig. 2). The nucleotide sequence of BB-15 cDNA was determined and compared with the published genome sequences of Australian IBDV strain 002-73 (11, 20). Nucleotide sequence homology of 94% was observed between the BB-15 and the 002-73 viruses at the 3′ end of genome segment B. Homology of the BB-15 sequence with other

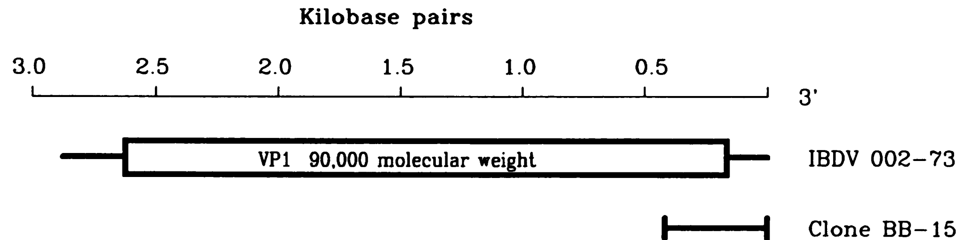

FIG. 3. Region of IBDV genome segment B represented by BB-15 cloned cDNA. Genome segment B of Australian IBDV isolate 002-73 is represented in the top line. This segment contains an open reading frame which is translated into the 90,000-molecular-weight viral polymerase (VP-1). The entire sequence of BB-15 cDNA is represented at the 3′ end of this genome segment.

regions of the 002-73 genome was not observed. This confirmed Northern blot hybridization results obtained with this clone and also identified its location on the 3′ end of this genome segment (Fig. 3).

Sensitivity and specificity of the BB-15 probe in dot blot hybridization reactions. The sensitivity of the BB-15 probe was determined by using purified viral RNA. After purification over CF-11 cellulose, BB viral RNA was quantitated by using spectrophotometry at 260 nm. Cell culture nucleic acid extracted from uninfected BGM-70 cells was included as a negative control. A volume of dimethyl sulfoxide was added to a final concentration of 90%, and the samples were denatured at 100°C for 3 min. Quantities ranging from 100 to 0.1 ng of denatured nucleic acids were spotted onto a nylon membrane which was then air dried and baked at 80°C for 1 h. Following 48 h of autoradiography, the BB-15 probe detected the lowest quantity of BB RNA tested, 0.1 ng (Fig. 4). This quantity of dsRNA represents approximately 1.4×10^7 IBDV particles. The ability of this probe to detect lower quantities of IBDV dsRNA was not determined.

The specificity of the BB-15 probe for IBDV was tested by using nucleic acid extracted from nine unrelated poultry viruses which included both DNA and RNA viruses. IBDV isolate UV was used as a positive control. Both the proteinase K and SSC-formaldehyde extraction procedures were used. The probe did not hybridize to nucleic acid extracted from any of the unrelated viruses tested; however, it did hybridize to the IBDV viral RNA from vaccine strain UV with both extraction procedures (data not shown).

Detection of IBDV dsRNA from infected cell culture samples. Five heterologous IBDV strains, which included four serotype 1 viruses and one serotype 2 virus, were grown in BGM-70 cells. The four serotype 1 viruses tested represent three different antigenic subtypes of IBDV. All five viruses were detected by the BB-15 probe (Fig. 5). This probe did not hybridize to control cell culture nucleic acid. Titers of the cell culture-propagated viruses ranged from $2 \times 10^{5.5}$ to $2 \times 10^{8.0}$ 50% tissue culture infective doses per ml (Fig. 5). Tenfold dilutions of the RNA samples were placed on the nylon membrane. Each sample contained a 25-, 2.5-, or 0.25-μl volume of the original cell culture medium. Differences in the intensities of the signals were observed among the viral RNA samples and appeared to correlate with virus titers in the original cell culture fluid.

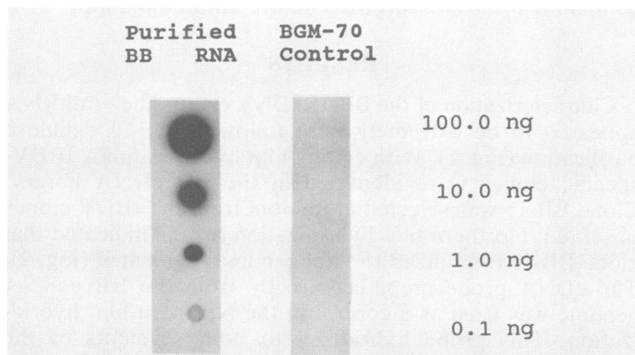

FIG. 4. Dot blot hybridization of the BB viral genome. A nick-translated probe was prepared from BB-15 cDNA and used to detect different concentrations of CF-11 cellulose-purified dsRNA from homologous IBDV isolate BB. Following 48 h of autoradiography, the lowest concentration (0.1 ng) of BB genomic RNA was detected. Nucleic acid from BGM-70 cells was used as a control.

Detection of IBDV dsRNA from tissue specimens. To determine the usefulness of the BB-15 probe and dot blot hybridization procedure for detecting IBDV in chickens, 4-week-old specific-pathogen-free chicks (COFAL/Marek; SPAFAS, Inc., Norwich, Conn.) were infected with the ST-C virus. At days 2 and 3 postexposure, the birds were sacrificed and bursa and spleen tissues were collected. Gross lesions typical of infectious bursal disease were observed in the bursas at necropsy.

Each sample contained a pool of two bursas or spleens homogenized in 2.0 ml of TNE buffer. IBDV genomic RNA was prepared for hybridization by using proteinase K, followed by extraction with phenol and chloroform. Tenfold dilutions of each sample placed on the nylon membrane contained a 250-, 25-, or 2.5-μl volume of the original tissue homogenate. Attempts to use the Nonidet P-40–SSC–formaldehyde extraction procedure on chicken tissue homogenates were unsuccessful because of the poor filterability of the samples.

The BB-15 probe detected IBDV in the spleens and bursas of experimentally infected birds at 2 and 3 days postexposure but not in similar tissues from mock-infected control chickens (Fig. 6).

Eight samples of bursa tissue were also obtained from five different commercial chicken operations designated A to E. Each sample contained a pool of 2 to 10 bursas which were homogenized in TNE buffer (1 ml per bursa). Samples containing 0.5-ml volumes of homogenized bursa tissue were treated with proteinase K as described previously and placed on a nylon membrane. A tenfold dilution of each sample placed on the membrane contained a 250-, 25-, or 2.5-μl volume of the original bursa homogenate. Dot blot hybridizations using the BB-15 probe indicated that bursa samples from farms A to D contained IBDV (Fig. 7). Hybridization of the BB-15 probe to samples from farm E and the bursa tissue from an uninfected control chicken was not detected. The presence of IBDV in the bursa tissue from farm C was confirmed by passage of the homogenate in BGM-70 cells.

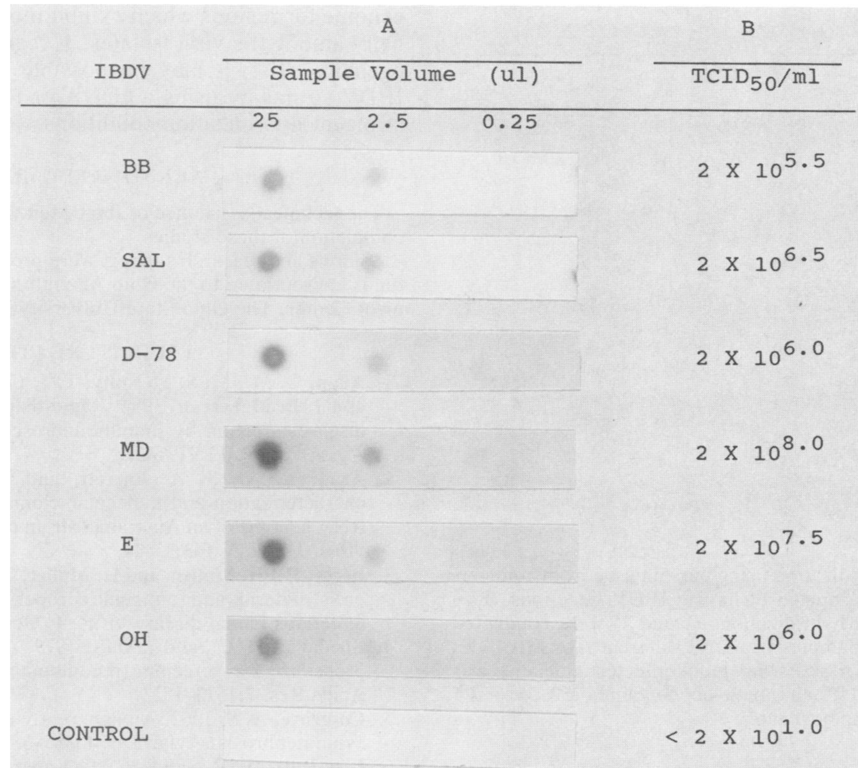

FIG. 5. Dot blot hybridization of cell culture-grown IBDV isolates using a nick-translated probe prepared from the BB-15 cDNA. (A) IBDV isolates were grown in BGM-70 cells. Viral RNA was extracted, and serial 10-fold dilutions of each sample were made. The resulting dilutions contained a 25-, 2.5-, or 0.25-μl volume of the original culture medium, which was placed on nylon membranes by using a dot blot filtration manifold. (B) The titer of each viral isolate is reported as the reciprocal of the last dilution which was equivalent to a 50% tissue culture infective dose ($TCID_{50}$). Mock-infected BGM-70 cells were used as a control.

Cytopathic effects typical of IBDV were observed after passage 2, and the IBDV isolate was subsequently identified by serologic procedures (N. M. Ismail and Y. M. Saif, Avian Dis., in press).

DISCUSSION

Diagnosis of IBDV infection currently relies on detection of antibodies to the virus and the presence of lesions in the bursa of Fabricius. Detection of IBDV infections is accomplished by using immunofluorescence techniques, electron microscopy, or in vitro virus neutralization, which requires isolation and growth of the virus in culture. We have shown that a dot blot hybridization procedure which uses cDNA probes can be used to detect genomic RNA of IBDV isolates as early as 2 days following exposure to the virus. Furthermore, the IBDV genomic RNA extracted directly from the bursas of infected birds can be detected with these probes, thereby eliminating the need for virus isolation and growth in culture. Bursa tissues submitted to our laboratory from five commercial poultry operations were tested with the BB-15 probe. The probe detected IBDV RNA in samples from four of the five farms. These studies demonstrate the potential for using nucleic acid probes for early diagnosis of IBDV infections in commercial poultry operations.

Our procedures for purification and cloning of the BB genome were generally similar to those used by Azad et al. (2) to clone Australian IBDV strain 002-73. However, several changes in purification of the dsRNA genome and second-strand cDNA synthesis reduced the number of steps

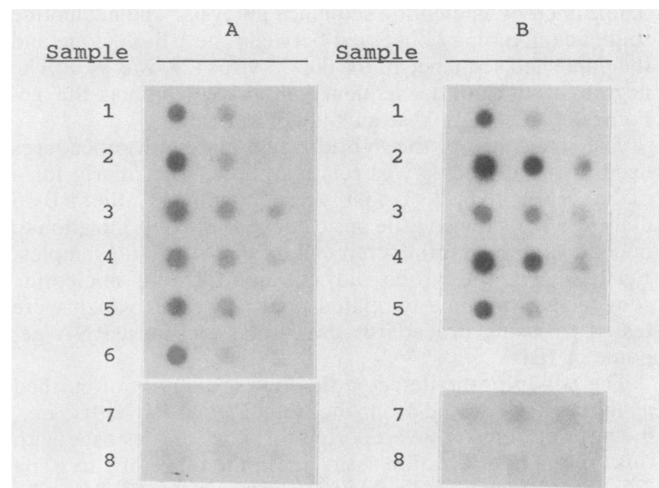

FIG. 6. Dot blot hybridization of IBDV (strain ST-C) extracted from spleen and bursal tissues. Two separate extractions and hybridizations (A and B) were conducted with the tissue samples. Four-week-old chickens were infected with ST-C, and tissues were collected at 2 or 3 days following exposure. Samples 1 and 2 were collected at days 2 and 3, respectively, from the spleen. Bursal samples 3 and 4 were collected at day 2, and bursal samples 5 and 6 were collected 3 days following exposure to ST-C. Samples 7 (spleen) and 8 (bursa) were collected from mock-infected control chickens on day 3. Each sample dot contained 250, 25, or 2.5 μl of the original tissue homogenate.

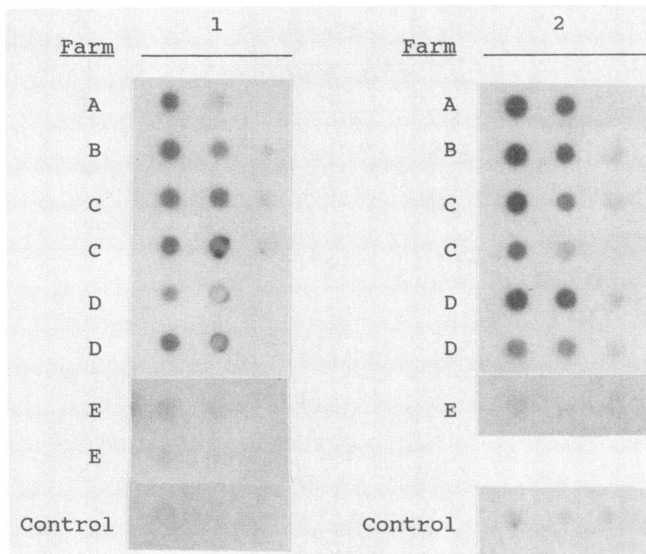

FIG. 7. Dot blot hybridization using bursal tissue from commercially reared chickens suspected of having IBDV infections. Two separate extractions and hybridizations (1 and 2) were conducted with the bursal tissues. Samples from five different farms (A to E) were examined. Bursal tissue from mock-infected chickens was used as a negative control. Each sample dot contained 250, 25, or 2.5 μl of the original bursa homogenate.

required to prepare IBDV clone libraries. Purification of viral dsRNA with CF-11 cellulose columns yielded high-quality RNA which could be used for cloning or probe synthesis. This procedure is easier and faster than differential LiCl purification of dsRNA (2, 6).

The Northern blot hybridization results indicated that clone BB-15 was located on IBDV genome segment B. The location of BB-15 on the 3′ end of genome segment B was determined by nucleotide sequence analysis. The nucleotide sequence homology observed between the BB-15 clone and the published sequence of the 002-73 virus (20) was 94%. The degree of nucleotide sequence homology among the genomes of other IBDV strains is not known.

The stringency of the hybridization and wash procedures used in these studies was relatively low, particularly for a cDNA-RNA hybrid. Under these conditions, the BB-15 cDNA probe showed little or no background hybridization to nucleic acid from uninfected cell culture or tissue samples. Furthermore, the probe did not hybridize to nucleotide sequences from nine unrelated poultry viruses which were tested by using procedures developed for the dsRNA genome of IBDV.

The BB-15 probe detected the lowest quantity of purified genomic RNA tested from the homologous BB virus, i.e., 0.1 ng. The sensitivities reported for other probes used with this type of hybridization assay are in the range of 1 to 10 pg (25, 26). Although the limits of sensitivity of the BB-15 probe were not determined, improvements in sensitivity may be possible by increasing the probe length or specific activity. In addition to detecting homologous virus, the BB-15 probe detected IBDV RNA extracted from four heterologous IBDV strains. These strains included IBDV isolates from three antigenic subtypes of serotype 1 virus and one serotype 2 virus. Thus, under relatively low-stringency hybridization conditions, the BB-15 probe showed broad specificity for the IBDV strains tested.

We are currently examining other areas of the IBDV genome for regions which exhibit more nucleotide heterogeneity among the viral isolates. If regions with greater heterogeneity exist, it may be possible to differentiate among IBDV strains by using a cDNA or RNA probe under more stringent hybridization conditions.

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LITERATURE CITED

1. **Allan, G. M., M. S. McNulty, T. J. Connor, R. M. McCracken, and J. B. McFerran.** 1984. Rapid diagnosis of infectious bursal disease infection by immunofluorescence on clinical material. Avian Pathol. **13**:419–427.
2. **Azad, A. A., S. A. Barrett, and K. J. Fahey.** 1985. The characterization and molecular cloning of the double-stranded RNA genome of an Australian strain of infectious bursal disease virus. Virology **143**:35–44.
3. **Becht, H., H. Muller, and H. Muller.** 1988. Comparative studies on structural and antigenic properties of two serotypes of infectious bursal disease virus. J. Gen. Virol. **69**:631–640.
4. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1523.
5. **Cosgrove, A. S.** 1962. An apparently new disease of chickens—avian nephrosis. Avian Dis. **6**:385–389.
6. **Diaz-Ruiz, J. R., and J. M. Kaper.** 1978. Isolation of viral double-stranded RNAs using a LiCl fractionation procedure. Prep. Biochem. **8**:1–17.
7. **Fahey, K. J., I. J. O'Donnell, and T. J. Bagust.** 1985. Antibody to the 32K structural protein of infectious bursal disease virus neutralizes viral infectivity in vitro and confers protection on young chickens. J. Gen. Virol. **66**:2693–2702.
8. **Grunstein, M., and J. Wallis.** 1979. Colony hybridization. Methods Enzymol. **68**:379–388.
9. **Gubler, U., and B. J. Hoffman.** 1983. A simple and very efficient method for generating cDNA libraries. Gene **25**:263–269.
10. **Huang, M. T. F., D. S. Manning, M. Warner, E. B. Stephens, and J. C. Leong.** 1986. A physical map of the viral genome for infectious pancreatic necrosis virus Sp: analysis of cell-free translation products derived from viral cDNA clones. J. Virol. **60**:1002–1011.
11. **Hudson, P. J., N. M. McKern, B. E. Power, and A. A. Azad.** 1986. Genomic structure of the large RNA segment of infectious bursal disease virus. Nucleic Acids Res. **14**:5001–5012.
12. **Jackwood, D. H., and Y. M. Saif.** 1987. Antigenic diversity of infectious bursal disease viruses. Avian Dis. **31**:766–770.
13. **Jackwood, D. J., Y. M. Saif, and J. H. Hughes.** 1982. Characteristics and serologic studies of two serotypes of infectious bursal disease virus in turkeys. Avian Dis. **26**:871–882.
14. **Jackwood, D. J., Y. M. Saif, and J. H. Hughes.** 1984. Nucleic acid and structural proteins of infectious bursal disease virus isolates belonging to serotypes I and II. Avian Dis. **28**:990–1006.
15. **Jackwood, D. H., Y. M. Saif, and J. H. Hughes.** 1987. Replication of infectious bursal disease virus in continuous cell lines. Avian Dis. **31**:370–375.
16. **Jonsson, L. G. O., and B. E. Engstrom.** 1986. Immunohistochemical detection of infectious bursal disease and infectious bronchitis viral antigens in fixed, paraffin-embedded chicken tissues. Avian Pathol. **15**:385–393.
17. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual p. 202–203, 466–467. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
18. **McNulty, M. S., G. M. Allan, and J. B. McFerran.** 1979. Isolation of infectious bursal disease virus from turkeys. Avian Pathol. **8**:205–212.
19. **Mellor, E. J. C., F. Brown, and T. J. R. Harris.** 1985. Analysis of secondary structure of the poly(C) tract in foot-and-mouth

disease virus RNAs. J. Gen. Virol. **66**:1919–1929.

20. **Morgan, M. M., I. G. Macreadie, V. R. Harley, P. J. Hudson, and A. A. Azad.** 1988. Sequence of the small double-stranded RNA genomic segment of infectious bursal disease virus and its deduced 90-kDa product. Virology **163**:240–242.
21. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling deoxyribonucleic acid to high specific activity in vitro by nick-translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
22. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5468.
23. **Shockley, L. J., P. A. Kapke, W. Lapps, D. A. Brian, L. N. D. Potgieter, and R. Woods.** 1987. Diagnosis of porcine and bovine enteric coronavirus infections using cloned cDNA probes. J. Clin. Microbiol. **25**:1591–1596.
24. **Tabor, S., and C. C. Richardson.** 1987. DNA sequence analysis with a modified bacteriophage T7 DNA polymerase. Proc. Natl. Acad. Sci. USA **84**:4767–4771.
25. **Tenover, F. C.** 1988. Diagnostic deoxyribonucleic acid probes for infectious diseases. Clin. Microbiol. Rev. **1**:82–101.
26. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA **77**:5201–5205.
27. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33**:103–119.