Molecular detection of bovine viral diarrhea virus in clinical samples by polymerase chain reaction-chemiluminscent DNA hybridization assay

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ABSTRACT

The polymerase chain reaction (PCR) followed by chemiluminescent probe hybridization assay was used for the detection of bovine viral diarrhea virus (BVDV) infections in cattle. BVDV RNA's from individual clinical specimens, from persistently infected (PI) and acutely diseased cattle were transcribed to cDNA using reverse transcriptase. Using a set of 18-mer oligonucleotide primers located within the conserved 5' untranslated region (UTR) of the BVDV genome, a 246 base pair target sequence from BVDV cDNA was successfully amplified by PCR. In dilution experiment, PCR analysis was at least 10 times more sensitive than BVDV isolation in detecting BVDV in serum from PI animals. The specificity and sensitivity of BVDV RNA detection by PCR amplification were confirmed by a chemiluminescent hybridization analysis of the amplified PCR products with BVDV-specific probes. The results suggest that PCR amplification-probe hybridization assay may be a useful addition in developing new rapid and sensitive tests for detection of BVDV. The speed and the sensitivity of this method might be of relevance for studies on epidemiological and pathogenesis of infection with BVD virus.

Key words: *Bovine viral diarrhea virus, detection, clinical samples, polymerase chain reaction, chemiluminescent hybridization.*

INTRODUCTION

Bovine viral diarrhea virus (BVDV) is an important pathogen that causes a variety of disease syndromes resulting in economic losses in domestic cattle herds (Meyling *et al.*, 1990). Infection with BVDV can result in at least three possible disease syndromes: bovine viral diarrhea, mucosal disease, and fetal disease (Brownlie, 1985).

Transmission may be vertical or horizontal, and may result in acute or persistent infection (Baker, 1987). Infection In utero may result in abortion, teratogenesis, stillbirths, or the birth of persistently infected (PI), immunotolerant animals (Kahrs, 1973; McClurkin et al., 1984).

Persistent infection is usually established by noncytopathic (NCP) biotypes of BVDV by infection of fetuses in utero before 110 to 120 days of gestation (Bolin et al., 1985 b; Brownlie et al., 1984). Mutation of NCP to a cytopathic (CP) biotype, or super infection with an antigenically related CP biotype in PI

animals, leads to the development of the usually fatal mucosal disease (Bolin et al., 1985 b and c; Brownlie et al., 1984). PI animals generally remain life-long virus carriers, shedding large quantities of virus in most bodily excretions and secretions (Duffell and Harkness, 1985; Roeder and Harkness, 1986). They are a significant source of infection to other cattle, thus perpetuating BVDV in the herd (Bolin et al., 1985 a; Werdin et al., 1989). Calves that are offspring of PI animals are also PI and immunotolerant to strains of BVDV that are antigenically similar to the persistently infecting strain (Baker, 1987).

Horizontally transmitted virus may result in diarrhea in young calves and the virus has been suggested to be immunosuppressive, having an auxiliary role in the establishment of opportunistic infections by other pathogens (Potgieter *et al.*, 1984).

BVDV is also a frequent contaminant in fetal bovine serum and other bovine products used in cell culture systems (Rossi et al., 1980). This infection presents a great problem for research laboratories and biological industries (Bolin *et al.*, 1991; Nuttall *et al.*, 1977).

Measures for prevention and control of BVDV infections center on the detection and removal of animals PI with BVDV and the prevention of transplacental infections (Bolin, 1990; Duffell and Harkness, 1984; Harkness, 1987). The techniques currently in use for isolation or detection of BVDV in contaminated cell cultures as well as in clinical samples from acutely or PI animals are virus isolation in cell culture followed by immunofluorescence or immunoperoxidase staining assays. These techniques are time-consuming and are too insensitive for detecting very low levels of infectious virus. Sensitive and novel approaches are needed to trace the spread and circulation of BVDV as well as to study the pathogenesis of the disease (Collect *et al.*, 1989). The polymerase chain reaction (PCR), using Taq DNA ploymerase of *Thermus aquaticus* has been shown to be a rapid and sensitive method for in vitro amplification of specific sequences of nucleic acids (Saiki *et al.*, 1988).

In a previous study, PCR amplification assay was used as a screening test using bulk tank milk samples for identifying dairy herds infected with BVDV (Radwan *et al.*, 1995). The aim of this study was to adapt PCR assay for the detection of BVDV in individual clinical samples and specimens using primers that amplify sequences near to the 5' end of the BVDV genome.

MATERIALS AND METHODS

Origin and processing of clinical specimens

Samples tested in this study were collected from 8 animals of the Holstein breed: two BVDV-PI heifers (#1 and #2), two adult lactating cows (#3 and #4), one PI bull (#5) produced by intravenous inoculation of a seronegative pregnant dam at day 90 of gestation with blood from PI animal #2 and one lactating cow acutely infected with BVDV by experimental intranasal inoculation (Brock and Radwan, 1993). Samples were also collected from 2 aborting cows (#1 and #2) that were from a herd with histories of abortion, weak calves and respiratory disease, suggestive of BVDV infection.

In order to estimate the diagnostic applicability of the PCR, samples of PI animals as well as of acutely diseased cattle were examined. The test materials consisted of serum, milk and semen from PI animals. From acutely diseased animals, white blood cell (WBC) preparations, milk and placental tissue samples were tested. Serum, milk and semen samples from BVDV-free animals were tested as negative controls. A summary of the

procedures performed on each category of sample is given in Table 1. Processed clinical samples from the PI and acutely diseased animals were examined in parallel by virus isolation and by PCR assay.

Conventional diagnostic procedures

Bovine turbinate (BT) cells were used for BVDV isolation and quantification according to the procedures described by Brock (1991). BT cell monolayers were inoculated with 1-ml amounts of clinical samples. NCP BVD virus isolation from the specimens was attempted after two consecutive passages, 4 days each. The CP BVDV NADL strain (obtained from Dr. S.R.Bolin, the National Animal Disease Center, USDA, Ames, IA, USA) and the NCP BVDV SD-1 strain (provided by Dr. K. V. Brock, Fac. Vet. Med, Auburn University, Auburn, Alabama, USA) were used and tested as reference. For BVDV quantification in serum samples, 50µl volumes of each10-fold sample dilutions in DMEM were inoculated onto cell monolayers in 96-well plates in replicates of 3. The results were assessed by indirect immunoperoxidase method (Afshar et al., 1989).

RNA extraction

Total RNA was isolated from one ml samples of BVDV infected cell culture suspensions or individual clinical samples using the lysis and RNA extraction procedures described by Chomczynski and Sacchi (1987). The extracted RNA was subjected to complementary DNA (cDNA) synthesis by reverse transcription.

Reverse transcription (RT)

In order to perform the RT reaction, the following reagents were added to 8.5 µl extracted RNA: 4 µl 5 X RT buffer (250 mM Tris-HCl [pH8.3], 375 mM KCl, 15 mM $MgCl_2$), 2 µl dithiothreitol (100 mM stocks), 2 µl deoxynucleotide triphosphates (10 mM stocks), 0.5 µl RNasin (40 units/µl stocks), 2 µl random hexamer primer (3 pg/ µl stocks), 1 µl M-MLV reverse transcriptase (200 units/ µl stocks). The reaction mixtures were incubated at 37°C for 1 hr, subsequently heat inactivated at 75°C for 10 min and the cDNA product was chilled on ice for immediate PCR testing or stored at -20°C.

PCR

The 18-bp primers 5'UTR 1 (5` GGC TAG CCA TGC CCT TAG 3`) and 5'UTR 2 (5` GCC TCT GCA GCA CCC TAT 3`) used for amplification of BVDV cDNA have a GC content of 60%. The oligonucleotide primers were designed based on the published NADL (Collect *et al.*, 1988), Osloss (Renard *et al.*, 1987) and SD-1 (Deng and Brock, 1992) BVDV nucleotide sequence data. The primers were synthesized and then purified at the Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Institute, Giza, Egypt. The 246-bp target is located between the bases 100 and 345 within the 5' UTR of BVDV genome.

Amplification of BVDV cDNA was performed as previously described (Radwan et al., 1995). Briefly, 8 µl GeneAmp® 10 X PCR buffer (500 mM KCl, 100 mM Tris-HCl [pH8.3]), 5 µl 5' UTR 1 and 5' UTR 2 primers (25 pmol/µl stocks), and 0.5µl AmpliTaq® DNA polymerase (5 units/µl stocks) and 2µl $MgCl_2$ (25 mM stocks) and nuclease-free water were added to the 20µl cDNA template in a total volume of 100 µl. The PCR mixture was initially denatured at 94°C for 4 min. PCR was allowed to run for 30 cycles in a DNA thermal cycler. Each cycle included three segments: template denaturation for 1 min at 94°C, primer annealing for 1.5 min at 55°C and primer extension for 3 min at 72°C. The

reaction mixture was further incubated at 72°C for 7 min as a final extension step. In each amplification round, a negative control reaction mixture of all reagents with no template added was used.

Sensitivity of BVDV detection by PCR in serum samples

A dilution experiment using serum samples from PI animal # 2 was done in order to determine the sensitivity of PCR amplification for detection of BVDV. Serum samples (contained 10^5CCID$_{50}$/ml) were serially diluted (10^{-1} to 10^{-5}) with serum from a BVDV-free cow in duplicate. One series was processed for RNA extraction, reverse transcription and PCR amplification as described above. The other series was processed for virus isolation for two passages followed by BVDV antigen detection as described above.

Analysis of the PCR products

a) Agarose gel electrophoresis

To visualize the yield, 10 µl of PCR products were subjected to electrophoresis at 100 V for 45 min on 1 % agarose gels in 1X TAE buffer. The gels were stained with 0.5µg/ml of ethidium bromide as described by Sambrook et al. (1989). The 1Kb ladder was used as a molecular weight marker to determine the length of the amplified fragment. The gels were examined under UV light and photographed using Polaroid instant films.

b) Southern blotting and Chemiluminescent hybridization

Following gel electrophoresis, the amplified DNA bands were blotted to 1.2 micron nylon membranes according to the procedures described by Southern (1975). The membrane filters were air dried and then baked at 80°C for one hr. The plasmid pBV-18 derived from BVDV-NADL (Contained inserts encompassing nucleotides 24-1308) was used to generate PCR-derived probes. Biotinylated probes from the amplified pBV-18 PCR products were made with the NEBlot® Phototope® Random Priming Labeling Kit (New England Biolabs, Beverly, MA, USA).

Blots were hybridized with biotinylated probes, as follows: each membrane was placed in a hybridization bag and thoroughly wet with 6x SSC (20x SSC = 3 M NaCl, 0.3 M NaCitrate, pH 7.0), followed by prehybridization with 6x SSC, 5x Denhardt's reagent (50x = 10 g Ficoll®-400, 50 g polyvinylpyrrolidone, 0.5 g bovine serum albumin in 500 ml water), 0.5% sodium dodecyl sulfate (SDS) and 100 µg/ml denatured salmon sperm DNA (0.1 ml of solution per cm^2 membrane) for 1 hr at 68°C. Five to seven microliters of each biotinylated probe were denatured in boiling water for 5 min, chilled on ice for 5 min, centrifuged briefly and then added to the prehybridization solution. Hybridization of the probe was allowed to proceed overnight at 68°C, with gentle rocking in a hybridization oven. After hybridization, the membrane was removed from the bag, washed twice in 2x SSC, 0.1% SDS at room temperature for 5 min each a then washed twice in 0.1x SSC, 0.1% SDS at 60°C for 15 min each. The washed membrane was placed in a hybridization bag for subsequent chemiluminescent detection

Phototope-Star detection was carried out using the Phototope®-Star Chemiluminescent Detection Kit (alkaline phosphatase/ streptavidin/CDP-Star®; New England Biolabs). Streptavidin, biotinylated alkaline phosphatase and CDP-Star reagents were sequentially added and removed from the bags, with wash steps in between each addition to remove excess reagent. At each step, the bag was rocked for 5 min at room temperature

with moderate agitation on a shaking rocker. After draining the final detection reagent, the membrane was sealed in the bag and exposed to Kodak X-ray film (Scientific Imaging Systems, [Eastman Kodak], New Haven, CT, USA) for 1-2 min, before the film was developed.

RESULTS

Parallel testing of clinical samples and specimens from acutely diseased and PI animals sampled in this study was done by virus isolation and by the PCR analysis. BVD virus was detected in all various samples collected from PI (serum, milk and semen) and experimentally-or naturally acutely infected animals (WBC preparation, milk and placental tissue homogenates). The virus titers in serum from PI heifers and cows #1 to #4 ranged from 10^4 to $10^{6.5}$ $CCID_{50}$ of BVDV/ml.

In this study, PCR oligonucleotide primers were synthesized to the highly conserved 5' UTR of BVDV genome to amplify specific sequences from BVDV. The primer pair of 5' UTR 1 and 5' UTR 2 produced a fragment of 246 bp after amplification.

RNA extracted from BT cells infected with the reference CP BVDV NADL and NCP BVDV SD-1 strains were tested with primer set in the PCR procedure. Fig. (1) shows the results of PCR amplification of BVDV cDNA from infected BT cell cultures. DNA fragment of 246 bp was resolved after agarose gel electrophoresis from cell cultures infected with NADL and SD-1 strains (Fig.1a; lanes 2 and 3, respectively). Uninfected BT cells from which RNA was extracted tested negative for BVDV (Fig.1a; lane 4). Moreover, PCR negative control sample with no cDNA template failed to produce amplification (Fig.1; lane 5).

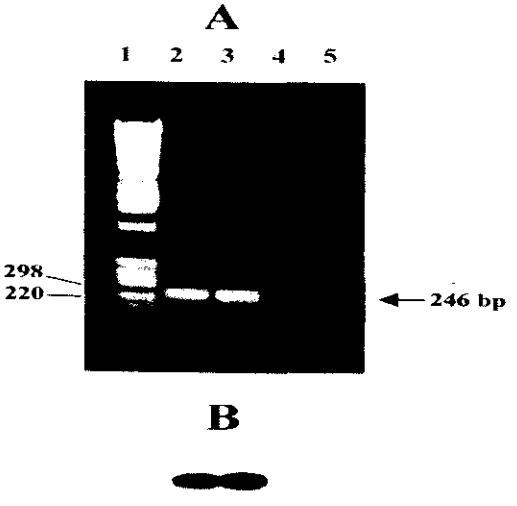

Fig. (1): A) Electrophoresis on 1% agarose gel of PCR amplified fragment from cell cultures infected with BVDV strains using 5' UTR 1 and 5' UTR 2 primers. 1Kb ladder (lane 1), BVDV strain NADL (lane 2), BVDV strain SD-1 (lane 3), uninfected BT cells (lane 4), PCR negative control mix with no cDNA template (lane 5). An arrow on the right indicates the 246-bp amplified product. Molecular weights of some marker fragments (lane 1) are indicated on the left. B) Southern chemiluminescent hybridization of PCR product DNA as described in (A) with the BVDV pBV-18 biotinylated probe.

Amplification of BVDV sequences was also achieved from clinical samples. Fig. (2) shows the results of BVDV cDNA amplification from serum, milk and semen samples of PI animals. BVDV RNA was detected by PCR analysis in serum samples

from PI heifers and adult lactating cows (Fig.2a; lanes 3-6). The difference in the intensity of PCR product DNA's from PI animals #1-#4 appears to reflect the differences in the titers of BVDV in the serum of these viremic animals. PCR amplification identified BVDV RNA extracted from milk samples collected from PI lactating animals #3 and #4 (Fig.2b; lanes 3 and 4). Amplified PCR product was also obtained from semen sample of the PI bull (#5) (Fig.2c; lane 2). Viral nucleic acid was not amplified from any of serum, milk and semen samples from BVDV-free animals used as negative controls.

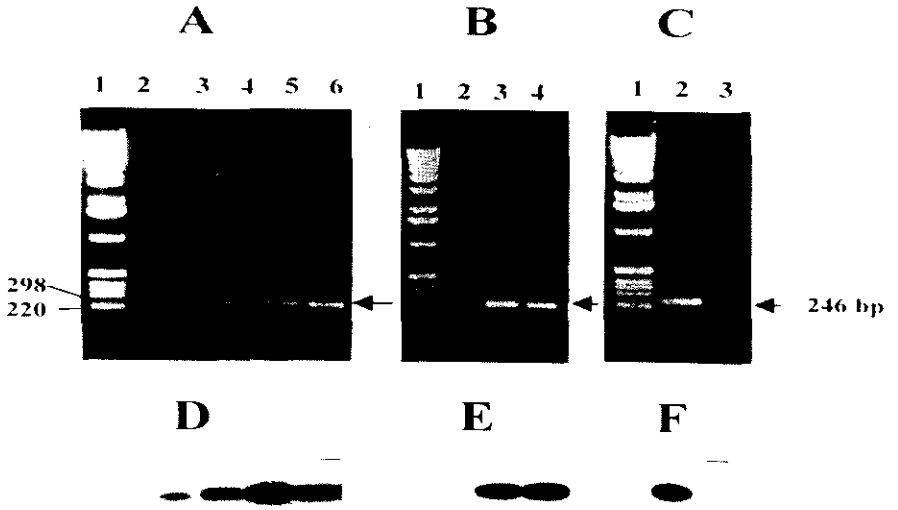

Fig. (2): A) Agarose gel electrophoresis of PCR amplification products of BVDV cDNA from samples of PI animals. A) Lanes 3-6, serum samples from PI heifers (#1 and #2) and adult lactating cows (#3 and #4), respectively; Lane 2, serum sample from BVDV-free animal. B) Lanes 3 and 4, milk samples from cows #3 and #4, respectively; lane 2, milk sample from a BVDV-free cow. C) Lane 2, semen sample from the PI bull (#5); lane 3, semen sample from a BVDV-free bull. In each panel, Lane 1 is the 1Kb ladder. An arrow on the right of each panel indicates the 246-bp amplified product. Molecular weights of some marker fragments (lane 1) are indicated on the left of each panel. D), E) and F) Southern chemiluminescent hybridization of PCR product DNA as described in (A), (B) and (C) with the BVDV pBV-18 biotinylated probe.

In the experimentally-acutely-infected cow, PCR amplification detected BVDV RNA extracted from milk and WBC preparations (Fig.3a; lanes 3 and 4, respectively). No specific amplification product was detected with cDNA from milk and WBC preparations from a BVDV-free cow used as negative controls (Fig. 3a; lanes 2 and 5, respectively). The PCR amplification allowed the detection of BVDV in the two placental tissue homogenates from naturally infected aborting cows #1 and #2 (Fig.4a; lanes 2 and 3).

Table (1). Summary of origin, processing and testing of clinical and field samples.

Origin	Sample	Processing	Test(s) performed
PI animals #1and #2 (heifers)	Clotted blood	5-10 ml-sample centrifuged to obtain 1 ml of serum	PCR; virus isolation and quantification
PI animals #3 and #4 (lactating cows)	-Clotted blood	5-10 ml-sample centrifuged to obtain 1 ml of serum	PCR; virus isolation and quantification
		1ml directly used	
	-Milk		
PI animal #5 (bull)	Semen	1ml directly used	PCR; virus isolation
Experimental- acutely infected Animal (lactating cows)	-EDTA blood	5-10 ml-sample centrifuged to obtain 1 ml of buffy coat	PCR; virus isolation
		1ml directly used	
			PCR; virus isolation
	-Milk		
Naturally- acutely infected Animals #1and #2 (aborting cows)	Placental tissue (100 mg)	Homogenized in DMEM medium to obtain 1 ml tissue homogenate	PCR; virus isolation

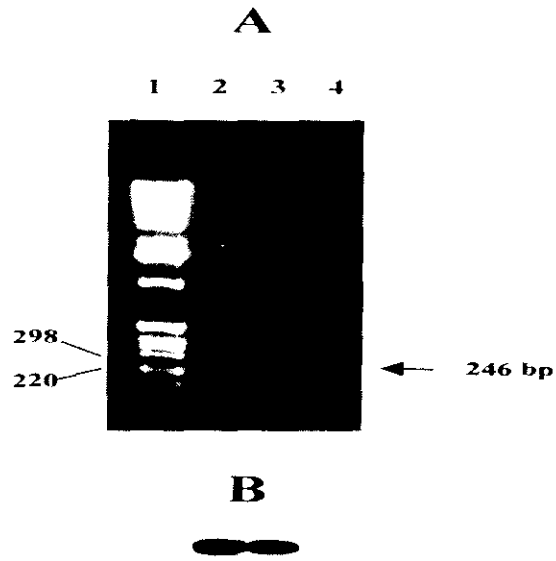

Fig. (3): A) Agarose gel electrophoresis of PCR amplification products of BVDV cDNA from samples of experimental-acutely-infected cow. Lane 1,1Kb ladder; lane 3, milk; lane 4, WBC preparation; lanes 2 and 5, milk and WBC preparation from a BVDV-free cow, respectively. An arrow on the right indicates the 246-bp amplified product. Molecular weights of some marker fragments (lane 1) are indicated on the left. B) Southern chemiluminescent hybridization of PCR product DNA as described in (A) with the BVDV pBV-18 biotinylated probe.

A dilution experiment was done in order to determine the sensitivity of BVDV RNA detection using PCR analysis with that of infectious virus isolation assay. In this experiment serum from PI heifer #2 was 10-fold serially diluted in duplicate with serum from a BVDV-free cow. One series was processed for RNA isolation and PCR analysis and the other series was used for infectious virus isolation assay. Up to a dilution of 10^{-3} (corresponds to 10^{2} $CCID_{50}$/ml), BVDV was still detected by both methods. However, only PCR analysis revealed the presence of BVDV RNA at a dilution of 10^{-4} (corresponds to 10 $CCID_{50}$/ml) (Fig. 5a and table 2). In this case, PCR analysis was 10 times more sensitive than virus isolation in detecting BVDV.

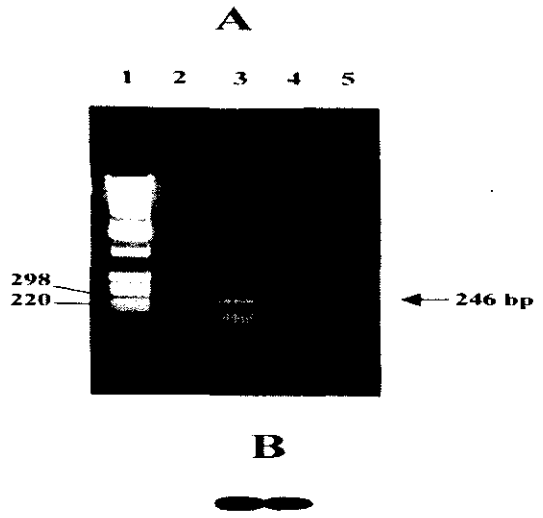

Fig. (4): A) Agarose gel electrophoresis of PCR amplification products from placental tissue specimens of naturally infected cows. Lane 1,1 Kb ladder; lanes 2 and 3, specimens from aborting animals #1 and #2, respectively; Lane 4, PCR negative control sample with no cDNA template. An arrow on the right indicates the 246-bp amplified product. Molecular weights of some marker fragments (lane 1) are indicated on the left. B) Southern chemiluminescent hybridization of PCR product DNA as described in (A) with the BVDV pBV-18 biotinylated probe.

Table (2). Sensitivity of PCR and infectious virus assay in the detection of BVDV in serum samples (, **, *** see materials & methods, for details).*

		Dilution**				
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Test	PCR***	+	+	+	+	-
	Virus assay	+	+	+	-	-

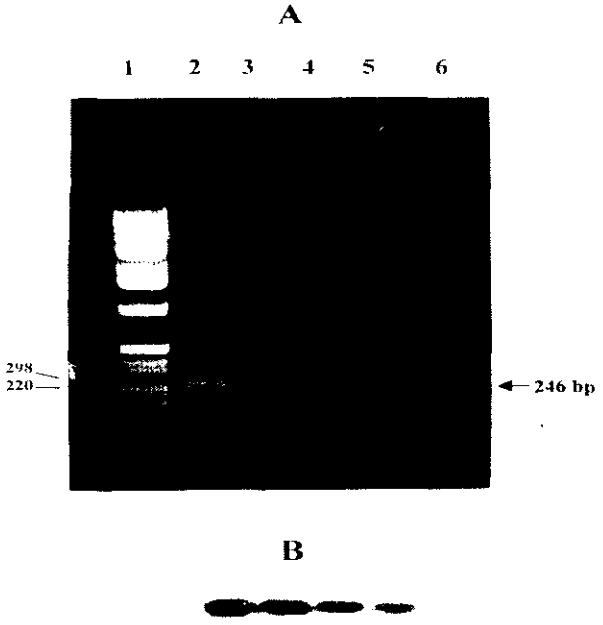

Fig. (5): Sensitivity of PCR in the diagnosis of BVDV. Serum from a BVDV-free cow was mixed with serum sample (105 CCID50/ml) from PI #2. RNA was isolated from 1 ml of each dilution, reverse transcribed and amplified. PCR products were size fractionated on agarose gel (A). Serum dilutions were from 10-1 to 10-5 in lane 2 to 6, respectively. An arrow on the right indicates the amplified product. Molecular weights of some marker fragments (lane 1) are indicated on the left. B) Southern chemiluminescent hybridization of PCR product DNA as described in (A) with the BVDV pBV-18 biotinylated probe.

The specificity and sensitivity of BVDV RNA detection by PCR amplification were confirmed by a chemiluminescent hybridization analysis of the amplified PCR products with BVDV-specific probes. The biotinylated probe reacted specifically with BVDV amplified product DNA and no hybridization signal was observed for BVDV-free negative control samples (Fig. 1b, Fig. 2d, e and f, and Fig. 3-5, b).

DISCUSSION

Bovine viral diarrhea virus is difficult to detect for several reasons. The virus, particularly the NCP grows slowly in cell culture and may require several passages before sufficient viral antigen is expressed to allow detection by antibodies. On the other hand, detection by antibody-based techniques is also difficult because of antigenic variation. In this study, a rapid, specific and sensitive PCR assay for detection of BVDV nucleic acid sequence was presented. The aim of the study was to apply PCR assay for the detection of BVDV RNA in cell culture and in individual clinical samples and specimens of infected animals. By this approach BVDV infection can rapidly and specifically diagnosed.

RNA was extracted from infected cell cultures and individual clinical samples using the potent protein denaturant guanidinium isothiocynate, which has been used to isolate intact biologically active RNA from RNase-rich tissues (Chomczynski and Sacchi, 1987). The use of this ingredient and protocol was efficient, fast, reliable and reproducible. Because BVDV is an RNA virus, it was necessary first reverse transcribe the viral RNA into single stranded cDNA, which was then used for the enzymatic amplification.

The oligonucleotide primer set used in this study was selected from regions of high nucleotide and amino acid conservation within the 5' UTR of BVDV genome (Letellier *et al.*, 1999). This study revealed that BVDV RNA could be detected in infected cells by reverse transcription followed by PCR amplification. Virus-specific amplification products of the expected sizes were found with both CP BVDV reference strain NADL and NCP BVDV strain SD-1 tested. The problem of BVDV contamination of cell lines, particularly with NCP strains, can be easily investigated using the technique described. It should be possible to screen batches of bovine sera for BVDV by PCR. Specific amplification products were consistently obtained from individual clinical samples and specimens from PI- and acutely diseased animals sampled in this study.

Because PCR permits the amplification of few BVDV particles, caution was exercised to avoid any possibility of generating false-positive results due to carry-over contamination of viral RNA or cDNA in any buffer or reagents. Proper negative controls were included in all PCR experiments.

During amplification of viral sequences, it is possible to get amplification of non-specific DNA (a fragment of an equivalent size to the expected specific band) in some samples. Therefore, the specificity and sensitivity of PCR amplification of BVDV cDNA from infected cell culture suspensions or individual clinical samples were confirmed by chemiluminescent hybridization analysis of the amplified PCR products with BVDV-specific probes.

There are several advantages of using PCR amplification to detect BVDV. The most important is the speed and sensitivity of the assay. Due to the sensitivity available using PCR, amplification can be done directly from samples obtained from PI- or acutely infected animals. Compared to viral isolation, PCR is

much faster, especially with the NCP biotype, which takes longer time to propagate and is the prevalent form in nature. The time required to obtain PCR results from the time of sample collection could be reduced to 24 hours. This significantly less time than minimal time required to obtain results by using standard viral isolation procedures. Amplification of BVDV cDNA directly from samples collected from infected animals provides the ability to characterize virus without the requirement of virus isolation in cell culture. Not surprisingly, the sensitivity of PCR virus detection method exceeded 10-fold that of virus isolation in cell culture. Similar results regarding the sensitivity of PCR for BVDV detection in bovine pooled serum were reported by Weinstock *et al.* (2001). Virus isolation method depends on the presence of infectious BVDV for detection. By contrast, the PCR detects viral nucleic acid even though the virus may have been inactivated.

In conclusion, RT-PCR could become useful for rapid detection of BVDV infections in cell culture and clinical materials from individual animals. Due to its exquisite sensitivity combined with the possibility for further analysis of amplified DNA products, RT-PCR-DNA hybridization assay could provide a powerful tool in studies on the epidemiology and pathogenesis of animal infections with BVD virus.

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الملخص العربي

الكشف الجزيئي عن إصابات فيروس مرض الإسهال الفيروسي البقري في الماشية باستخدام اختبار تفاعل إنزيم البلمرة المتسلسل ومسبر تهجين الحمض النووي غير المشع

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استخدم اختبار تفاعل إنزيم البلمرة المتسلسل والتهجين مع مسبر الحمض النووي غير المشع للكشف عن إصابات فيروس مرض الإسهال الفيروسي في الماشية.
تم النسخ العكسي للحمض النووي الريبوزي المستخلص من العينات الإكلينيكية من حيوانات دائمة الإصابة بالفيروس و آخرى مصابة به إصابة حادة إلي الحمض النووي الدايوكسي ريبوزي المكمل (cDNA) بواسطة إنزيم النسخ العكسي. و باستخدام زوج من بادئات التفاعل مكون من ١٨ نونيدة تم اختياره من منطقة قرب نهاية الـ ٥ غير المترجمة لبروتين بجينوم الفيروس تم بنجاح مضاعفة منطقة طولها ٢٤٦ زوجا من القواعد مستهدفة من الحمض النووي الدايوكسي ريبوزي المكمل الخاص بالفيروس بواسطة هذا الاختبار. و لقد كانت حساسية اختبار تفاعل سلسلة إنزيم البلمرة تفوق مثيلتها بإجراء طرق عزل الفيروس علي مزارع الخلايا بأكثر من ١٠ مرات للكشف عن الفيروس في عينات سيرم من حيوانات دائمة الإصابة بالفيروس. وقد تم التأكد من تخصص و حساسية اختبار تفاعل إنزيم البلمرة المتسلسل في الكشف عن الحمض النووي لفيروس مرض الإسهال الفيروسي بإجراء اختبار التهجين مع مسبر الحمض النووي غير المشع المحضر من إحدى العترات القياسية من هذا الفيروس.
و قد دلت النتائج علي أن تفاعل إنزيم البلمرة يمكن أن يمثل إضافة جديدة في تطبيق اختبارات سريعة و حساسة للكشف عن الفيروس، كما يمكن الإفادة من سرعة و حساسية هذا الاختبار في دراسات علي وبائية و كيفية حدوث و تطور الإصابة بفيروس مرض الإسهال الفيروسي في الماشية.