APPLICATION OF THE POLYMERASE CHAIN REACTION TO THE DETECTION OF BOVINE VIRAL DIARRHEA VIRUS IN CLINICAL SAMPLES FROM INFECTED CATTLE

BY

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ABSTRACT

The polymerase chain reaction (PCR) assay was used for the detection of bovine viral diarrhea virus (BVDV) infections in cattle. BVDV RNA's from a cytopathic BVDV reference strain, a noncytopathic strain and 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 results suggest that PCR amplification 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 epidemilogy and pathogenesis of infection with BVD virus.

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 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 (Bolin et al, 1985 b; Brownlie et al., 1984) by infection of fetuses in utero before 110 to 120 days of gestation. 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).

The aim of this study was to adapt PCR assay for the detection of BVDV in cell culture and in clinical samples and specimens using primers that amplify sequences near to the 5' end of the BVDV genome.

MATERIALS AND METHODS

Viruses and cell culture.

The CP BVDV reference strain NADL and NCP BVDV strain SD-1 were tested in this study. The NADL strain was obtained from Dr. S.R.Bolin, the National Animal Disease Center, USDA, Ames, IA. The SD-1 was isolated from a PI heifer in South Dakota. BVDV strains were cultured on bovine turbinate (BT) cells. These cells were determined to be free of NCP BVDV contamination by indirect fluorescence antibody staining with BVDV-specific antibodies. Cells were grown in Dulbecco's minimum essential medium (DMEM) supplemented with 10% horse serum. Confluent monolayers of BT cells were inoculated with an appropriate BVDV strain using 0.1 multiplicity of infection and incubated for 2-3 days (NCP strain) or 5-7 days (NCP) at 37°C under humidified 5% CO_2 atmosphere.

Origin and processing of clinical specimens:

Samples tested in this study were collected from 7 animals of the Holstein breed: 2 BVDV-PI heifers (#1 and #2), 2 adult lactating cows (#3 and #4), 1 PI bull (#5) produced by intravenous inoculation of a seronegative pregnant dam at day 90 of gestation with blood from PI animal #2 and 1 lactating cow acutely infected with BVDV by experimental intranasal inoculation (Radwan et al., 1995). Animals were housed in an isolation facility during the study. 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 table of the procedures performed on each category of sample is shown in Table 1. Serum samples were obtained from whole blood collected from PI heifers and lactating cows. Milk samples were collected from the PI lactating cows as wells from the experimentally infected cow following milking using a bucket milker assembly. Semen samples were obtained from the PI bull. In order to obtain WBC from the experimentally infected cow, whole EDTA blood samples were centrifuged at 1,000 g for 30 minutes at room temperature. The buffy coat layer was then aspirated and mixed with 2 volumes of 0.83% NH_4Cl and incubated at room temperature for 10 minutes. The mixtures were centrifuged at room temperature and precipitates were then washed twice in PBS at room temperature. Precipitates were resuspended in DMEM. Placental tissue specimens (100 mg) from the 2 aborting cows were homogenized in plastic bags in a lab stomacher in DMEM at approximately 10% w/v suspension, treated with penicillin (100 units/ ml), streptomycin (100 μg/ml) and fungizone (2 μg/ml). The tissue homogenates were allowed to stand at 4°C for 1-2 hours and were then centrifuged at 2,000 g to clarify.

Processed clinical samples from the PI and acutely diseased animals were examined in parallel by virus isolation and/or quantification and by PCR assay.

Conventional diagnostic procedures and virus quantification:

BT cell monolayers were inoculated with 1-ml amounts of clinical samples as described above for BVDV cell culture. NCP BVD virus isolation from the specimens was attempted after 2 consecutive passages, 4 days each. For BVDV quantification in serum and milk samples, 50 μl volumes of each 10-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 BVDV infected BT cells and clinical samples using the lysis and RNA extraction procedures described by Chomczynski and Sacchi (1987). Briefly, 1-ml amount of cell culture suspension or clinical sample was mixed with 5 ml of guanidium solution (4 M guanidium isothiocyanate, 25 mM sodium citrate [pH 7.0], 0.5% w/v sarcosyl, and 0.1 M 2-mercaptoehanol). Subsequently, 0.5 ml of 2 M sodium acetate (pH 4.0), 5 ml of saturated phenol and 1 ml of chloroform-isoamyl alcohol (24:1) were added. The mixture was vortexed for 10 seconds, cooled on ice for 15 minutes, and then centrifuged at 10,000 g for 20 minutes at 4°C. RNA retained in the resulting aqueous phase was precipitated with an equal volume of isopropanol, and kept at -20°C overnight. The precipitated RNA was pelleted by centrifugation at 10,000 g for 30 minutes at 4°C and the RNA pellet was then vacuum dried in a SpeedVac centrifuge. The dried pellet was resuspended in 8.5μl of 0.2 diethyl pyrocarbonate treated ultrapure water and subjected to cDNA synthesis by reverse transcription.

Reverse transcription (RT):

In order to perform the RT reaction, the following reagents were added to the 8.5μl RNA: 4 μl of 5 X RT buffer (250 mM Tris-HCl [pH8.3], 375 mM KCl, 15 mM $MgCl_2$), 2 μl of dithiothreitol (100 mM), 2 μl of deoxynucleotide triphosphates (10 mM stocks), 0.5 μl of RNasin (40 units/ μl), 2 μl of random hexamer primer (3 μg/ μl stocks), 1 μl of M-MLV reverse transcriptase (200 units/ μl) and the extracted RNA. The reaction mixtures were incubated at 37°C for 1 hour, subsequently heat inactivated at 75°C for 10 minutes 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 and 5'UTR 2 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, Agricultural Research Institute, Giza, Egypt. The 246-bp target is located between bases 100 and 345 within the 5' UTR of BVDV genome. There are 2 recognition sites for the restriction enzyme Taq I at bases 203 and 225 (Fig. 1).

Amplification of BVDV cDNA was performed as previously described (Radwan et al., 1995). Briefly, 8 μl of GeneAmp® 10 X PCR buffer (500 mM KCl, 100 mM Tris-HCl [pH8.3]), 5 μl of 5' UTR 1 and 5' UTR 2 primers (25 μmol/ μl), and 0.5 μl of AmpliTaq Gold™ DNA polymerase (5 units/μl; Perkin-Elmer Corp., USA) and 2μl of $MgCl_2$ (25 mM) and nuclease-free water were added to the 20-μl cDNA template in a total volume of 100 μl. A layer of 50 μl of mineral oil was added to prevent evaporation of the reagents. The PCR mixture was initially denatured at 94°C for 9 minutes. PCR was allowed to run for 30 cycles in a DNA thermal cycler (Perkin-Elmer Cetus Corp., USA). Each cycle included 3 segments: template denaturation for 1 minute at 94°C, primer annealing for 1.5 minutes at 55°C and primer extension for 3 minutes at 72°C. The reaction mixture was further

incubated at72°C for 7 minutes as a final extension step. In each amplification round, a negative control reaction mixture of all reagents with no template added was used.

Analysis of the PCR products:

To visualize the yield, 10 µl of PCR products were subjected to electrophoresis at 100 V for 45 minutes on 1 % agarose gels in 1X TAE buffer. The gels were stained with 0.5 µg/ml 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 type 57 Polaroid instant films.

The specificity of PCR amplification of BVDV cDNA from placental tissues obtained from the 2 naturally infected aborting cows was verified by the restriction digestion of the PCR products. PCR product (10µl) was added to 5 µl of the appropriate 10X enzyme buffer and 2 units of Taq I enzyme (New England Biolabs, Inc., Beverly, MA, USA) as recommended by the manufacturer. Samples were electrophoresed under the conditions described above. Digested PCR product from NADL BVDV cDNA was simultaneously subjected to electrophoresis in order to control the digestion by the Taq I enzyme.

Sensitivity of BVDV detection by PCR in serum samples:

A dilution experiment using serum samples from PI animal # was done in order to determine the sensitivity of PCR amplification for detection of BVDV. Serum samples (contain 10^5 $CCID_{50}$/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 2 passages followed by BVDV antigen detection as described above.

RESULTS

Parallel testing of clinical samples and specimens from acutely diseased and PI animals sampled in this study was done by virus isolation and/or quantification 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. Milk from the PI lactating cows #3 and #4 contained 10^5 and 10^6 $CCID_{50}$/ml, respectively while milk from the experimentally-acutely infected cow contained $10^{2.5}$ $CCID_{50}$/ml.

In this study, PCR oligonucleotide primers were synthesized to the highly conserved 5' UTR of BVDV genome (fig.1) 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 CP BVDV strain NADL and NCP BVDV strain SD-1 were tested with primer set in the PCR procedure. Fig.2 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.2a; lanes 2 and 3, respectively). Uninfected BT cells from which RNA was extracted tested negative for BVDV (Fig.2a; lane 4). Moreover, PCR negative control sample with no cDNA template failed to produce amplification (Fig.2a; lane 5). The specificity of PCR amplification for detection of BVDV was verified by amplification of BVDV cDNA insert encompassing nucleotides 24 to 1308 cloned in pGEM plasmid (pBV-18) used as a positive control. The pBV-18 plasmid yielded the specific 246-bp product DNA fragment (fig.2b; lane 2).

Amplification of BVDV sequences was also achieved from clinical samples. Fig.3 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.3a; 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 levels of viremia. PCR amplification identified BVDV RNA extracted from milk samples collected from PI lactating animals #3 and #4 (fig.3b; lanes 3 and 4). Amplified PCR product was also obtained from semen sample of the PI bull (#5) (fig.3c; lane 2). Viral nucleic acid was not amplified from any of serum, milk and semen samples from BVDV-free animals used as negative controls.

In the experimentally-acutely-infected cow, PCR amplification detected BVDV RNA extracted from milk and WBC preparations (fig.4; 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.4; lanes 2 and 5 respectively).

PCR primers 5' UTR 1 and 5' UTR 2 used in this study allowed detection of BVDV in the 2 placental tissue homogenates from naturally-infected aborting cows #1 and #2 (fig.5; lanes 2 and 3). The specificity of PCR amplification of BVDV cDNA from the 2 naturally infected aborting cows was confirmed by restriction enzyme Taq I digestion of the PCR products. From the NADL and SD-1 sequences, it was predicted that internal Taq I sites in the amplified fragment would result in 2 major fragments of 103 and 120 bp after digestion (see fig.1). Specific cleavage by the restriction enzyme Taq I of PCR products from placental tissue homogenates yielded a pair of fragments of expected sizes (fig.6; lanes 3 and 4). PCR product from BVDV strain NADL that was used to control Taq I digestion gave an identical yield (fig.6; lane 2).

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.7 and table 2). In this case, PCR analysis was 10 times more sensitive than virus isolation in detecting BVDV.

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 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 clinical samples using the potent protein denaturant guanidinium isothiocynate, which has been used to isolate intact biologically active RNA from RNase-rich tissues (Chomcynski 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 (Lettellier 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 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 of PCR amplification of BVDV cDNA from placental tissues obtained from the 2 naturally infected aborting cows was verified by the restriction digestion of the PCR products. Only BVDV-specific amplified sequences contains the expected internal restriction site. Upon digestion with Taq I, two fragments of expected sizes (120 and 103 bp) were resolved by electrophoresis. Further confirmation of the

specificity was obtained from an identical electrophoresis pattern of Taq I digested PCR product for BVDV reference strain NADL.

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 is 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 that of virus isolation in cell culture 10-fold. 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. Due to its exquisite sensitivity combined with the possibility for further analysis of amplified DNA products, RT-PCR could provide a powerful tool in studies on the epidemiology and pathogenesis of infection with BVD virus.

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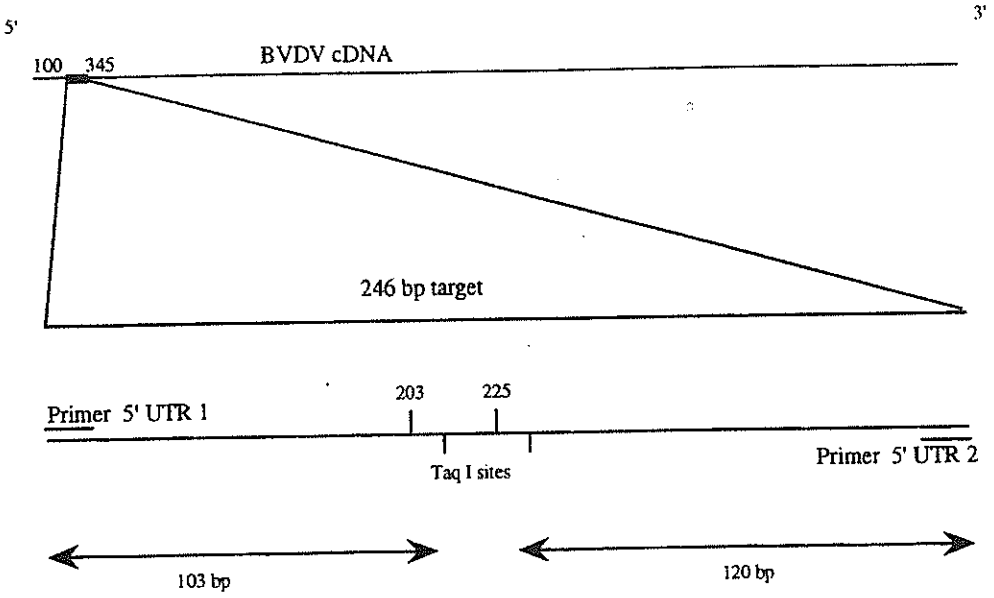
Table 1. Summary of origin, processing and testing of clinical and field samples

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

Table 2. Sensitivity of PCR and infectious virus assay in the detection of BVDV in serum samples*

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

*From PI heifer # 2.
**Undiluted serum contained 10^5 $CCID_{50}$ infectious BVDV particles/ml. Dilutions were made from a BVDV-free cow.
*** RNA extracted from 1 ml of serum was used for reverse transcription and PCR analysis. PCR products were resolved on 1% agarose. The presence or absence of the BVDV specific amplification product is indicated with "+" or "-" respectively.



PRIMER 5' UTR 1 GGC TAG CCA TGC CCT TAG

PRIMER 5' UTR 2 GCC TCT GCA GCA CCC TAT

Fig. 1. Illustration of the 246-bp amplification target from BVDV cDNA. Restriction enzyme Taq I cuts the target into 2 major fragments, 103 and 120 bp long.

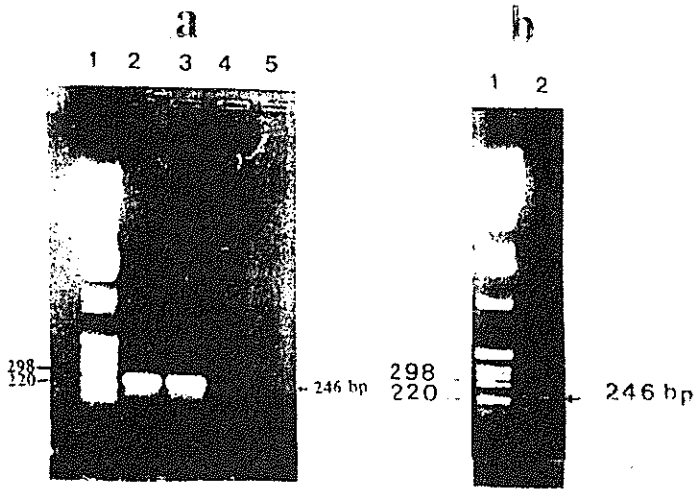


Fig.2. Electrophoresis on 1% agarose gel of PCR amplified fragment from cell cultures infected with BVDV strains using 5' UTR 1 and 5' UTR 2 primers. From left to right: lane 1, 1Kb ladder; Lane 2, BVDV strain NADL; lane 3, BVDV strain SD-1; Lane 4, uninfected BT cells; lane 5, PCR negative control mix 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.

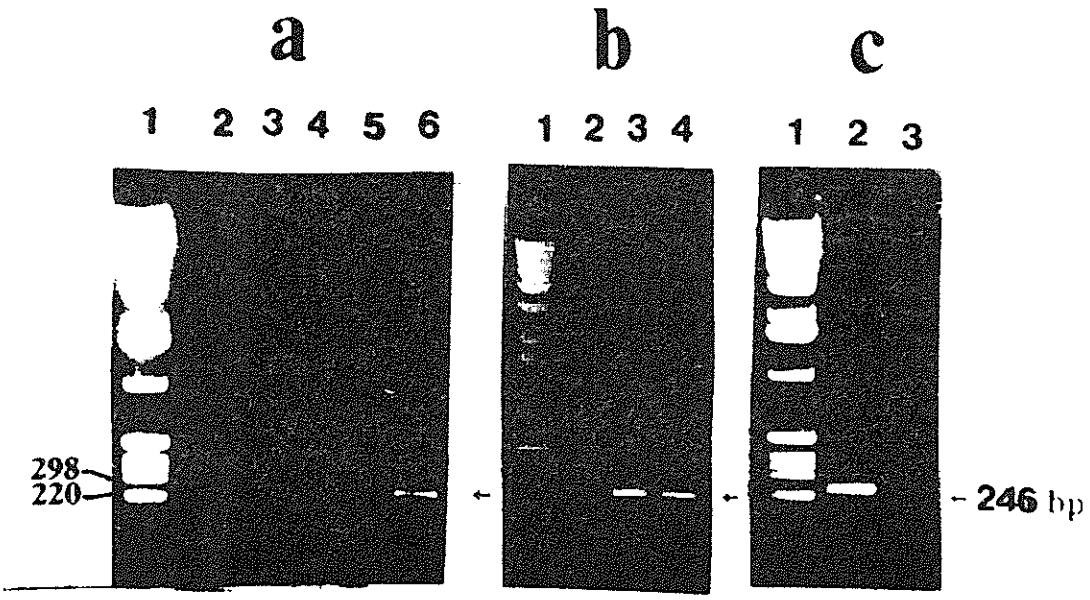


Fig.3. Agarose gel electrophoresis of PCR amplification products of BVDV cDNA from samples of PI animals. A) Lanes 3-6, serum samples from PI heifers (#1 and #2) and adult lactating cows (#3 and #4), respectively; Lane 2, serum sample from BVDV-free animal. B) Lanes 2 and 3, milk samples from cows #3 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.

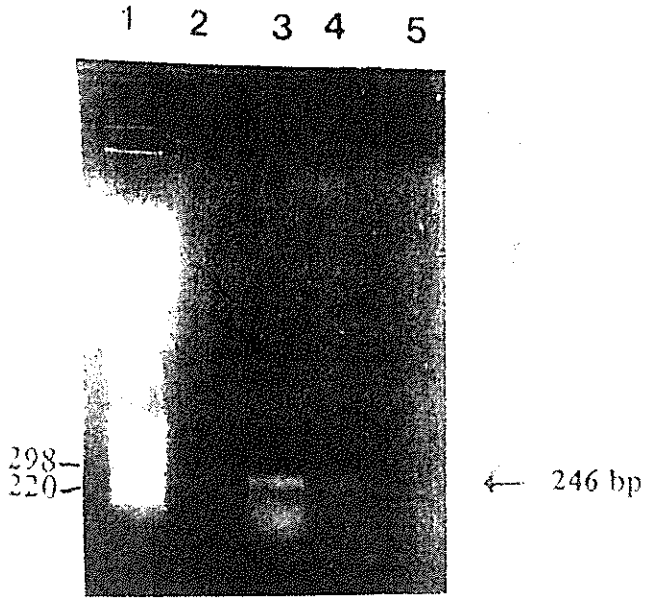


Fig.4. Agarose gel electrophoresis of PCR amplification products of BVDV cDNA from samples of experimental-acutely-infected cow. Lane 1, 1Kb ladder; lane 3, milk; lane 4, WBC preparation; lanes 2 and 5, milk and WBC preparation from a BVDV-free cow respectively. An arrow on the right indicates the 246-bp amplified product. Molecular weights of some marker fragments (lane 1) are indicated on the left.

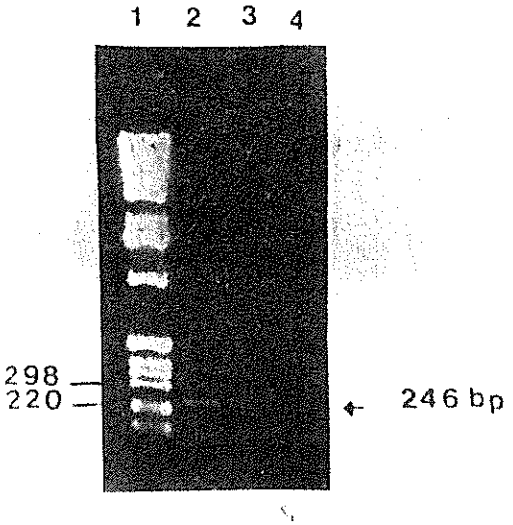


Fig. 5. Agarose gel electrophoresis of PCR amplification products from placental tissue specimens of naturally infected cows. Lane 1, 1 Kb ladder; lanes 2 and 3, specimens from aborting animals #1 and #2, respectively; Lane 4, PCR negative control sample with no cDNA template. An arrow on the right indicates the 246-bp amplified product. Molecular weights of some marker fragments (lane 1) are indicated on the left.

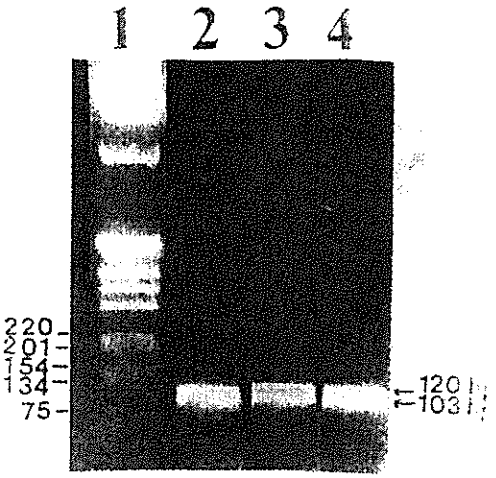


Fig.6. Agarose gel electrophoresis of the 246-bp amplified fragment after digestion with restriction enzyme Taq I. Lane 1, 1 Kb ladder; lane 3, BVDV strain NADL; lanes 4 and 5, placental tissue specimens from naturally-infected aborting cows #1 and #2, respectively. Molecular weights of some marker fragments are indicated on the left. Arrows on the right indicate the 120- and 103-bp fragments.

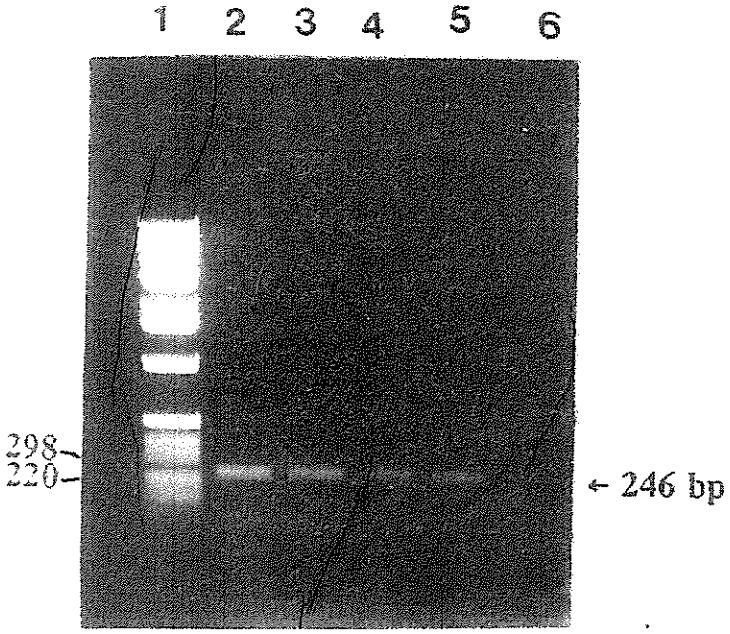


Fig.7. Sensitivity of PCR in the diagnosis of BVDV. Serum from a BVDV-free cow was mixed with serum sample (10^5 $CCID_{50}$/ml) from PI #2. Serum dilutions were from 10^{-1} to 10^{-5} in lane 2 to 6, respectively. RNA was isolated from 1 ml of each dilution, reverse transcribed and amplified. PCR products were size fractionated on agarose gel. An arrow on the right indicates the amplified product. Molecular weights of some marker fragments (lane 1) are indicated on the left.

الملخص العربي

تطبيق اختبار تفاعل سلسلة إنزيم البلمرة للكشف عن إصابات فيروس مرض الإسهال الفيروسي البقرى في الماشية

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**قسم الباثولوجيا البيولوجية – كلية الطب البيطري – جامعة أوبرون – أوبرون، الاباما – الولايات المتحدة الأمريكية.

استخدم اختبار تفاعل سلسلة إنزيم البلمرة للكشف عن إصابات فيروس مرض الإسهال الفيروسي في الماشية حيث تم نسخ الحمض النووي الريبوزى المستخلص من فيروسات مرجعية مختلفة من حيث تأثيرها الباثولوجي على خلايا الزرع النسيجي وكذلك من العينات الإكلينيكية والحقلية من حيوانات دائمة الإصابة بالفيروس وأخرى مصابة إصابة حادة بواسطة إنزيم النسخ العكسي. وباستخدام زوج من بادئ التفاعل مكون من ١٨ نيكلوتيده تم اختياره من منطقة قرب نهاية الـ ٥ غير المترجمة لبروتين بجينوم الفيروس تم مضاعفة منطقة طولها ٢٤٦ زوج من القواعد مستهدفة من الحمض النووي الديوكسي ريبوزى المكمل الخاص بالفيروس بواسطة هذا الاختبار. ولقد كانت حساسية اختبار تفاعل سلسلة إنزيم البلمرة تفوق مثيلتها بواسطة إجراء طرق عزل الفيروس على الزرع النسيجي بـ ١٠ مرات للكشف عن الفيروس في عينات سيرم من حيوانات دائمة الإصابة بالفيروس. وقد دلت النتائج على أن اختبار تفاعل سلسلة إنزيم البلمرة يمكن أن يمثل إضافة جديدة في تطبيق اختبارات سريعة وحساسة للكشف عن الفيروس، كما أن سرعة وحساسية هذا الاختبار يمكن الإفادة منها في دراسات على وبائية وكيفية حدوث وتطور الإصابة بفيروس مرض الإسهال الفيروسي البقرى في الماشية.