Use of polymerase chain reaction to simultaneously detect and type bovine viral diarrhoea viruses isolated from clinical specimens


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Summary
The techniques of indirect immunofluorescence (IF), immuno-peroxidase (IP) staining and the one-step reverse transcriptase polymerase chain reaction (RT-PCR) were compared for detection of 102 isolates of bovine viral diarrhoea virus (BVDV) in infected cell cultures. The BVDV was obtained from bovine clinical specimens, including sera, buffy coats and tissues, submitted from farms located in the States of Iowa and Wisconsin, United States of America. The IF technique detected 88/102 (86.3%) of the viral isolates, whereas IP staining detected an additional 4 isolates (92/102; 90%). The one-step RT-PCR using primers derived from the 5' untranslated region of the BVDV genome detected 102/102 (100%) of the BVDV isolates. A second-round PCR utilising another pair of PCR primers from the 5' untranslated region, allowed rapid genotyping of BVDV. The procedure used showed that the PCR assay based on the 5' untranslated region of the virus genome is the most sensitive indicator for BVDV detection in cell culture, and is also of considerable epidemiological importance since it allowed rapid genotyping of BVDV isolated from clinical specimens. In addition to detection and genotyping of BVDV isolated from clinical specimens, the RT-PCR procedure can be used for routine screening of locally produced and imported biologicals for BVDV contamination. However, the procedure requires further refinement to enable direct application on the clinical specimen.

Keywords

Introduction
Bovine viral diarrhoea virus (BVDV) is a small enveloped RNA virus currently classified in the genus Pestivirus of the family Flaviviridae (35), together with classical swine fever (hog cholera) and border disease virus of sheep. All pestiviruses are serologically related (36). The BVDV genome consists of a single-stranded RNA molecule of positive polarity containing approximately 12.5 kilobases (kb) with a single large open reading frame (ORF) that encodes a polypeptide of...
mature viral proteins by cellular and viral proteases (13). The ORF is preceded by 360 to 385 nucleotides constituting the 5' untranslated region (UTR), which is the most conserved region in the BVDV genome (18, 32).

Two biotypes of BVDV exist: cytopathic BVDV (cpBVDV) and non-cytopathic BVDV (ncpBVDV). The cpBVDV induces cytoplasmic vacuolation and cell death in susceptible cell cultures (22), whereas the ncpBVDV has little effect in cell cultures (30). Either separately or in combination, both viral biotypes induce diseases that range from clinically mild to the fatal syndrome, mucosal disease (2, 5, 10). Primary post-natal infections with BVDV, termed 'bovine viral diarrhoea' (BVD), are usually subclinical but may result in fever, lymphopaenia, inappetence, diarrhoea and drop in milk yield (2, 38). The ncpBVDV is usually isolated from field cases of this disease. However, certain ncpBVDV strains induce a clinically severe form of acute BVD that is characterised by fever, lymphopaenia, thrombocytopenia, diarrhoea, thrombocytopenic purpura and death (7, 15, 16). Primarily, BVDV genotype 2 has been associated with clinically severe outbreaks of acute BVD (37, 45). Infection of seronegative cows during early pregnancy (first trimester) with ncpBVDV can result in abortion, stillbirth, mummification, congenital anomalies, weak calves and calves born immunotolerant to, and persistently infected with, BVDV (19, 33, 38). Persistently infected cattle serve as reservoirs for maintaining and spreading BVDV within a herd. Upon superinfection with certain cpBVDV, persistently infected animals succumb to fatal mucosal disease or chronic BVD (5, 10). Most naturally occurring outbreaks of mucosal disease appear to be induced by antigenically matched pairs of ncp- and cpBVDV (14, 28). However, antigenically distinct viruses also pair to induce mucosal disease (43). Mucosal disease and chronic BVD are rare and usually occur in cattle between six months and two years of age. These diseases are of low morbidity, and are characterised by diarrhoea, wasting and lameness. Mucosal disease has a rapid onset and short duration, while chronic BVD develops slowly over several weeks to months (2, 20, 38).

The genomic nucleotide sequences for several BVDV strains are available (12, 17, 42, 46). This information has been used to develop nucleic acid probes to the viral RNA for detection of BVDV by hybridisation (9). The recent progress of primer-directed nucleic acid amplification using the polymerase chain reaction (PCR) provides a powerful, rapid and sensitive tool for the detection of specific nucleic acid sequences of pestiviruses, and PCR is more sensitive and less laborious than the hybridisation assays (3, 8, 9, 44). In a recent study, BVDV isolates were segregated into two distinct genotypes (BVDV 1 and BVDV 2), based on sequences of the 5' UTR using PCR assays (45).

Control measures for BVDV depend mainly on maintaining closed herds, thorough screening of animals for BVDV infection prior to entering the herds, and vaccination (4, 24, 38).

The discrimination between genotypes of BVDV by the classical virological and serological means is cumbersome due to the antigenic relatedness of the viruses.

The work presented here describes use of a PCR assay that detects both cytopathic and non-cytopathic isolates of BVDV in cell culture. The procedure allows simultaneous genotyping of BVDV isolated from clinical specimens using the 5' UTR of the BVDV genome as target.

**Materials and methods**

**Viruses**

Two strains of BVDV type 1: the NADL strain (a cytopathic strain) and the New York-1 (NY-1) strain (a non-cytopathic strain); and the Parker strain of BVDV type 2 (a non-cytopathic strain) were used as control viruses in this study. The three BVDV strains were propagated and titrated on bovine turbinate (BT) cells.

**Clinical specimens submitted for detection of bovine viral diarrhoea virus**

Clinical bovine specimens (n = 134), each representing one animal, were collected from 134 male and female cattle of different ages and breeds (Hereford, Holstein, Angus, Brown Swiss and cross-bred). The clinical specimens used in this study were submitted from farms located in Iowa and Wisconsin in the United States of America (USA) for routine diagnosis of BVD in the form of sera, buffy coat cells (from ethylene diamine tetra-acetic acid [EDTA]-anticoagulated blood), nasal swabs, tissues such as placenta, lung, trachea, small intestine, colon, spleen, kidney and mesentric lymph node. The clinical signs reported included fever, depression, emaciation, inappetence, anaemia, icterus, diarrhoea (watery or ropy and tinged with mucous or blood), straining, dehydration, cough, pneumonia, mucoid occulo-nasal discharges, small erosions or ulcers in the mouth or around the nostrils, cracking of the hooves with involvement of the coronary band, recumbency, failure of conception or abortion at late pregnancy. The prominent post-mortem findings were erosions, ulcers, haemorrhages, congestion and necrotic lesions throughout the intestinal tract, congestion, haemorrhages and necrotic lesions in the spleen, and the lung was often congested, with necrotising interstitial bronchopneumonia. Some animals had been vaccinated against BVDV either once or twice with commercially available vaccines, such as modified live infectious bovine rhinotracheitis virus (IBRV), parainfluenza-3 virus (PI-3V), BVDV, bovine respiratory syncytial virus (BRSV), or Pasteurella or Haemophilus vaccines, or with killed IBRV, BVDV, PI-3V, BRSV, Haemophilus or Pasteurella vaccines.
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Cell culture and virus isolation

BVDV-free BT cells were grown in minimum essential medium with Earle’s salts (MEME) supplemented with 10% horse serum. Before starting the experimental studies, BT cell cultures were passaged five to ten times and tested for BVDV contamination by indirect immunofluorescence (IF) (34) and immunoperoxidase (IP) staining (6). To avoid cross-contamination of samples, clean and sterile tools, utensils and vehicles were used for each sample.

The clinical specimens were processed for virus isolation following the routine procedure used at the Veterinary Diagnostic Laboratories, Iowa State University, USA. A representative specimen from the tissue submitted for diagnosis was cut, minced with sterile scissors, homogenised in a pre-labelled stomaching bag in a Stomacher and the entire contents were centrifuged at 9,000 × g for 20 min at 4°C. The supernatants were collected into sterile pre-labelled centrifuge tubes and kept at −70°C. Blood samples were obtained either as serum or EDTA anti-coagulated blood. The serum samples centrifuged at 1,000 × g for 10 min at room temperature. The EDTA-blood samples were centrifuged at 1,000 × g for 30 min at room temperature and the buffy coats were isolated and diluted in Earle’s balanced salt solution as needed. Processed materials from clinical specimens were inoculated onto confluent BT cell monolayers grown in 24-well plates. This was achieved as follows: the cell culture growth medium (MEME, 10% horse serum) was aspirated from all the wells. The cell monolayers were then washed once with serum-free medium and 0.45 ml per well of each clinical sample was inoculated into duplicate wells. Two wells per plate were kept as non-infected cell controls and the plates were incubated at 37°C in a humidified 5% CO₂ atmosphere. The plates were observed daily for cytopathic effect (CPE). After two blind passages in BT cells for two successive weeks, culture supernatants were harvested to be examined by IF, IP staining and PCR.

Identification of bovine viral diarrhoea virus isolates in cell culture by immunoperoxidase staining

The IP staining procedure described by Bolin et al. (6) was used with some modification. Briefly, flat-bottom 96-well microtitration tissue culture plates seeded with confluent BT cells were inoculated in duplicate with 0.05 ml of clarified samples of culture supernatant harvested from the 24-well plates inoculated with materials from clinical specimens. Two rows in each microtitration plate were used as non-infected cell controls. The BT cells were washed with PBS (pH 7.6); fixed in cold acetone, rewashed with PBS and reacted with anti-BVDV polyclonal antiserum (diluted 1:100 in binding buffer; PBS, pH 7.6 containing 0.02% Tween-20 and 3% NaCl) for 1 h at room temperature. Then, BT cells were washed with PBS (pH 7.6), reacted to peroxidase conjugated recombinant protein G, diluted in binding buffer to 1:1,000 for 30 min at room temperature and washed with PBS (pH 7.6). Finally, freshly prepared substrate solution (3-amino-9-ethyl carbazol) was added to cells. A red-brown stain appeared within 5 to 15 min in the cytoplasm of infected cells. A lack of staining in infected cells was considered to indicate a negative result.

Indirect immunofluorescence for detection of bovine viral diarrhoea virus

The IF test was performed following the procedure described (34) with some modification. Briefly, flat-bottom 96-well tissue culture microtitration plates, pre-seeded with BT cells, were inoculated in duplicate with 0.05 ml per well of clarified samples of culture supernatant harvested from the 24-well plates inoculated with materials from clinical specimens. Two rows in each microtitration plate were used as non-infected cell controls. The BT cells were washed with PBS (pH 7.6); fixed in cold acetone, rewashed with PBS and reacted with anti-BVDV polyclonal antiserum (diluted 1:100 in PBS). Then, BT cells were washed with PBS (pH 7.6) and probed with fluorescein isothiocyanate-labelled antibovine immunoglobulin G (diluted to 1:50 in PBS). Microtitration plates were washed in three changes of PBS, mounted with glycerol (4:1 in PBS) and examined microscopically for greenish-yellow fluorescence using a fluorescence microscope. A sample was considered to show positive results when at least two virus-specific fluorescent cells were observed in any microtitration plate well.

Isolation of total RNA from bovine viral diarrhoea virus-infected cell cultures

Confluent monolayers of BT cells were inoculated with BVDV strains NADL, NY-1 and Parker at 100 tissue cell infective dose 50 (TCID₅₀), and incubated at 37°C until CPE appeared in 80% to 90% of the cells inoculated with NADL strain (usually for 36-48 h), or for 4 to 5 days for cells inoculated with NY-1 and Parker strains of BVDV. Total cellular RNA was extracted from virus-infected cells using the method described by Chomezynski and Sacchi (11). The cell monolayers were removed from the culture flask by scraping and lysed in ten volumes (v) of denaturing solution (4M guanidium thiocyanate; 25 mmol sodium citrate, pH 7.0; 0.5% N-Lauroyl sarcosine and 0.1M 2-mercaptoethanol). The lysate was mixed thoroughly with 0.1 v of 2M sodium acetate (pH 4.0), 1 v of saturated phenol (pH 4.5) and 0.2 v of chloroform/isooamyl alcohol (49:1, v/v), and incubated on ice for 15 min. The suspension was centrifuged for 20 min at 14,000 × g at 4°C and RNA was precipitated from the aqueous phase by addition of 1 v of 100% isopropanol, incubation for 30 min at −20°C, and centrifugation at 14,000 × g for 10 min at 4°C. The RNA pellet was washed in 75% ethanol and dried at room temperature. The RNA pellet was then dissolved in diethyl pyrocarbonate (DEPC)-treated sterile double-distilled water and stored at −70°C until used.
In a separate procedure, the viral RNA was isolated using a modified process described by Lanciotti et al. (29). The clarified samples of culture supernatant harvested from the 24-well plates inoculated with materials from clinical specimens were thoroughly mixed with an equal volume of 2X denaturing solution, 0.1 v of 2M sodium acetate (pH 4.0), 1 v of equilibrated phenol, and 0.2 v of chloroform/isoamyl alcohol (49:1; v/v). The mixture was incubated on ice for 15 min and centrifuged at 14,000 X g for 15 min. The RNA was precipitated, washed, dried and dissolved as described before. Concentration of RNA in final preparation was calculated by spectrophotometric analysis as described by Sambrook et al. (47).

**Reverse transcription-polymerase chain reaction assay**

A reverse transcription-PCR (RT-PCR) assay using two sets of oligonucleotide primers spanning the 5' UTR of the BVDV genome was performed on RNA isolated from cell cultures inoculated with processed clinical specimens.

**Selection of the oligonucleotide primers**

Primers were designed according to the published complementary DNA (cDNA) sequence data of BVDV strain NADL (12) and strain 890 (46), with the aid of a commercial sequence analysis computer program.

**Set A**

Sense 5' GTA GTC GTC AGT GGT TCG 3' (188-205)
Antisense 5' GCC ATG TAC AGC AGA GAT 3' (369-386).

**Set B**

Sense 5' CGA CAC TCC ATT AGT TGA GG 3' (191-210)
Antisense 5' GTC CAT AAC GCC ACG AAT AG 3' (277-296).

Set A was chosen from the 5' UTR of BVDV strain NADL and was used for the first round PCR (one-step RT-PCR) to detect RNA of BVDV in samples. Set B was chosen from the 5' UTR of a BVDV genotype 2 (strain 890) and was used for the second round PCR to identify genotypes 1 and 2 of BVDV.

**First-round polymerase chain reaction (one-step reverse transcriptase-polymerase chain reaction)**

The first-round RT-PCR conditions were used with primer set A. The reaction was performed in a total volume of 50 µl containing: 1 X PCR buffer (20 mmol Tris-HCl pH 8.4; 50 mmol KCl); 1.5 mmol MgCl2; 0.2 mmol deoxyribonucleotide triphosphates mixture; 100 pmol of each primer; 2.5 units (U) Thermus aquaticus (Taq) polymerase; 200 U of M-MLV (Moloney murine leukaemia virus) reverse transcriptase; 7.5 U of ribonuclease (RNase) Inhibitor; 0.2-0.4 pg RNA sample; and RNase-free (DEPC-treated) sterile dd H2O up to 50.0 µl. The samples were then subjected to the following thermocycling in a programmable thermocycler:

One cycle of:
- 42°C for 1 h then 94°C for 3 min.
- 94°C for 30 s
- 50°C for 45 s
- 72°C for 1 min.

One cycle of:
- 72°C for 10 min.

**Second-round polymerase chain reaction**

The second-round reaction was prepared similarly to the first-round reaction (in a 50 µl total volume reaction) but RT enzyme and RNase inhibitor were omitted and primer set B was used instead of set A. First-round amplification product (2-3 µl) was used as the template for the second round PCR. Conditions for the second round PCR were as follows:

One cycle of:
- 94°C for 2 min.
- 94°C for 30 s
- 50°C for 45 s
- 72°C for 1 min.

One cycle of:
- 72°C for 7 min.

Results

**Isolation of bovine viral diarrheoa virus from clinical specimens**

Results of BVDV isolation from clinical specimens revealed that 32/134 of the specimens were free from BVDV by all tests used in this study and 20 of the viral isolates produced a CPE in BT cell cultures (Table I). The other 82 viral isolates were non-cytopathic in BT cell cultures.

**Immunodetection of bovine viral diarrheoa virus in cell cultures after virus isolation**

Following viral isolation, the IF and IP staining techniques were used to detect BVDV antigen in BT cell cultures. IF detected 88 of the BVDV isolates (Table I). The other 82 viral isolates were non-cytopathic in BT cell cultures.

**Detection of bovine viral diarrheoa virus viral RNA by the polymerase chain reaction**

The analysis of amplification products using agarose gel electrophoresis and ethidium bromide staining indicated that
amplified DNA obtained from 102 viral isolates (Table I) was of the expected size. Amplified DNA from the one-step RT-PCR using primers derived from 5’ UTR of the NADL strain of BVDV genotype 1 was approximately 198 base pairs (bp) for all laboratory BVDV strains tested (NADL, NY-1 and Parker) (Fig. 1) and for 102/102 of BVDV isolates (Fig. 2a). In the second-round PCR, which used the primers selected from 5’ UTR of the 890 strain of BVDV genotype 2, the electrophoretic migration of the amplified products corresponded to the expected 105 bp fragment size for 31/102 of the BVDV isolates that had given positive results with first-round RT-PCR (Fig. 2b).

### Discussion

In this study, bovine clinical samples (sera, buffy coats and tissue specimens) were submitted from different locations in the States of Iowa and Wisconsin, USA, for the diagnosis of bovine viral diarrhoea-mucosal disease. Briefly, BVDV was isolated by long-term cultivation on BT cell cultures, followed by verification of identity by immunodetection using the IF and IP staining methods. Of 134 clinical specimens tested, 102 specimens gave positive results for BVDV, while 32 specimens gave negative results for BVDV in all tests used in this study. Only 20/102 (19.6%) of the viral isolates were cpBVDV, while 82/102 (80.4%) isolates were ncpBVDV. Immunofluorescence detected 88/102 (86.3%) of the viral isolates whereas the IP staining detected 92 viral isolates (90%).

### Table I

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<th>IP</th>
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<th>2nd PCR (b)</th>
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<td>27</td>
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<td>82</td>
<td>88</td>
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BVDV : bovine viral diarrhoea virus  
ncp : non-cytopathic  
BVDV biotype cp : cytopathic  
IF : immunofluorescence  
IP : immunoperoxidase staining  
PCR : polymerase chain reaction  
RT-PCR : reverse transcriptase-polymerase chain reaction

a) Detected any genotype (1 and/or 2) of BVDV isolates  
b) Detected only genotype 2 of BVDV isolates  
c) A variety of tissues mixed together prior to processing (lung, trachea, oesophagus, liver, spleen, kidney, foetal placenta, heart and brain)

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**Fig. 1**
Agarose gel electrophoresis of polymerase chain reaction (PCR) amplicons derived from the 5’ untranslated region of bovine viral diarrhoea virus (BVDV) genotype 1 and genotype 2

Agarose gel (1.5%) stained with ethidium bromide
Lanes:
1: 100 bp DNA ladder
2-8: viral isolates from clinical specimens

The arrow refers to a unique size of the amplified cDNA fragments of approximately 198 base pairs (bp).

**Fig. 2a**
Agarose gel electrophoresis of the first-round polymerase chain reaction (PCR) amplicons derived from 5' untranslated region of bovine viral diarrhoea virus isolates from clinical specimens.

Agarose gel (1.5%) stained with ethidium bromide.

The one-step RT-PCR, which used the 5' UTR primers, detected BVDV from 102 of 134 of the clinical specimens tested in the first round of testing. The second-round PCR using primer set B of the 5' UTR identified 31/102 (30%) of the viral isolates as BVDV genotype 2. These findings support the claim that the 5' UTR is useful for both molecular detection and genotyping of the BVDV strains/isolates (4, 23, 25, 26, 39, 45).

The comparative detection of BVDV isolated from clinical specimens indicated that the RT-PCR based on the 5' UTR of the virus genome was the most sensitive indicator for detection of virus. This method also enabled differentiation of BVDV genotypes, which can be of considerable epidemiological importance and might be of value in control programmes. However, direct detection of the virus in crude clinical samples by RT-PCR is often unsuccessful. In this study, the authors attempted RT-PCR using known viral positive and viral negative clinical specimens and were not successful. This might be due to either the presence of certain elements that are inhibitory to reverse transcriptase or Taq polymerase enzymes in the clinical samples or to masking of the target template by proteins coagulated during denaturation of nucleic acids in the clinical specimen (1, 40). Therefore RT-PCR, although a very powerful and sensitive tool for detection of BVDV, requires further work before the method can be directly applied to clinical specimens.

The lower sensitivity of immunodetection for isolated viruses compared to the PCR might be related to either poor growth of the virus or presence of toxic elements in the samples (27). Also, the polyclonal antiserum used as a primary antibody in both IF and IP tests might react inefficiently with certain antigenic variants of BVDV. There have been reports that some BVDV strains react poorly with antibodies made against antigenically heterologous viral strains (21, 34, 41). The PCR detects both viable and non-infectious viral particles (dead or immature) since it identifies specific nucleotide sequences which are generally more stable in a clinical sample (31).

In conclusion, the results obtained in this study proved that the one-step RT-PCR is a highly sensitive, non-radioactive tool for detection of the BVDV in cell culture. It is also fast, and results could be obtained within 48 hours. Moreover, the RT-PCR based on the 5' UTR of the viral genome is of great epidemiological and pathological importance as 30% of the isolates detected in this study were of BVDV genotype 2. In addition to detection of virus isolated from clinical specimens, the RT-PCR procedure can be used for routine screening of locally produced and imported biologicals for BVDV contamination.

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Application de la technique d’amplification en chaîne par polymérase à la recherche et au typage simultanés des virus de la diarrhée virale bovine à partir de prélèvements cliniques


Résumé
Les auteurs présentent les résultats d’une étude comparée portant sur la capacité des techniques d’immunofluorescence indirecte (IF), de coloration par immunoperoxydase (IP) et d’amplification en chaîne par polymérase-transcriptase inverse (reverse transcription-polymerase chain reaction : RT-PCR) à passage unique, pour reconnaître 102 isolats du virus de la diarrhée virale bovine (DVB) dans des cultures cellulaires infectées. Le virus de la DVB a été obtenu à partir de prélèvements cliniques effectués sur des bovins (séums, couches leucocytaires et tissus) provenant de divers élevages de l'Iowa et du Wisconsin, États-Unis d’Amérique. L’IF a détecté 88/102 (86,3 %) isolats du virus alors que la coloration IP en a reconnu quatre de plus (92/102 : 90 %). La technique RT-PCR à passage unique, utilisant des amorces provenant de la région 5’ non traduite du génome du virus de la DVB, a détecté tous les isolats. Grâce à une deuxième amplification, utilisant une autre paire d’amorces PCR provenant de la région 5’ non traduite, le génome du virus de la DVB a pu être typé rapidement. La procédure utilisée montre non seulement que l’épreuve PCR, basée sur la région 5’ non traduite du génome du virus, est l’indicateur le plus sensible du virus de la DVB en culture cellulaire, mais aussi qu’elle est d’une grande utilité en épidémiologie, dans la mesure où elle permet de procéder à la détermination rapide du gènotype du virus de la DVB isolé dans des prélèvements cliniques. Outre la détection et la détermination du gènotype du virus de la DVB à partir de prélèvements cliniques, la méthode RT-PCR peut être utilisée en routine en vue de déterminer une éventuelle contamination par le virus de la DVB des produits biologiques fabriqués localement ou importés. Toutefois, ce procédé doit encore être perfectionné avant d’être appliqué directement aux prélèvements cliniques.

Mots-clés
Uso de la reacción en cadena de la polimerasa para detectar y a la vez tipificar virus de la diarrea viral bovina aislados en muestras clínicas


Resumen
Se compararon las técnicas de inmunofluorescencia indirecta (IF), tinción por inmunoperoxidasa (IP) y transcripción inversa-reacción en cadena de la polimerasa (reverse transcription-polymerase chain reaction: RT-PCR) para la detección de 102 aislados del virus de la diarrea viral bovina (VDVB) en cultivos celulares infectados. El virus se obtuvo a partir de muestras de bovinos con síntomas clínicos, básicamente sueros, capas leucocitarias y tejidos procedentes de granjas de los estados de Iowa y Wisconsin, en Estados Unidos de América. La técnica de IF permitió detectar un 86,3% (88/102) de las cepas víricas, mientras que la tinción por IP detectó las mismas y otras cuatro (92/102, o un 90%). La aplicación de una RT-PCR de un paso utilizando secuencias cebadoras de la región 5' no traducida del VDVB permitió detectar el 100% (102/102) de los aislados víricos. Utilizando otro par de cebadores procedentes de la región 5' no traducida, una segunda ronda de PCR permitió genotipar rápidamente el VDVB. Al margen de su notable importancia epidemiológica por la rapidez con que permitió tipificar el genoma de los VDVB presentes en muestras clínicas, el procedimiento utilizado puso de manifiesto que la prueba de PCR basada en la región 5' no traducida del genoma vírico constituye el indicador más sensible para detectar el VDVB en cultivo celular. Además de la detección y tipificación genómica de VDVB presentes en muestras clínicas, la prueba de RT-PCR puede aplicarse a la detección de la contaminación por el VDVB de productos biológicos tanto locales como importados. Sin embargo, es preciso perfeccionar el método antes de que sea posible aplicarlo directamente sobre la muestra clínica.

Palabras clave

References


