Frequent detection of bovine polyomavirus in commercial batches of calf serum by using the polymerase chain reaction

Rob Schuurman, 1 Bert van Steenis, 2 Ans van Strien, 1 Jan van der Noordaa 1 and Cees Sol 1

1 Department of Virology, Academic Medical Centre, University of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam and 2 National Institute of Public Health and Environmental Protection, P.O. Box 1, 3720 BA Bilthoven, The Netherlands

Twenty commercial batches of calf serum, obtained from several suppliers, were tested for the presence of bovine polyomavirus (BP\(\text{y}^+\)V) DNA and antibodies against the virus. Using polymerase chain reaction (PCR) technology, BP\(\text{y}^+\)V DNA was detected in 70% of the batches; no BP\(\text{y}^+\)V was detected in any of the negative control samples. The specificity of the amplification reactions was proven by hybridization. PCR results were confirmed by virus isolation experiments performed with five PCR-positive and five PCR-negative serum batches. The results indicate that the use of calf serum to supplement tissue culture media involves a serious risk of contaminating cell cultures with BP\(\text{y}^+\)V. No correlation was observed between the presence or absence of anti-BP\(\text{y}^+\)V immunoglobulins and the detection of BP\(\text{y}^+\)V-specific DNA sequences in the serum batches.

Introduction

The complete nucleotide sequence of the dsDNA genome of bovine polyomavirus (BP\(\text{y}^+\)V) has been reported recently (Schuurman et al., 1990; DDBJ/EMBL/GenBank accession number D00755). The genome of BP\(\text{y}^+\)V consists of a circular DNA molecule of 4697 nucleotides, which means that BP\(\text{y}^+\)V contains the smallest genome of those polyomaviruses which have been sequenced.

BP\(\text{y}^+\)V was formerly known as the CK isolate of stump-tailed macaque virus (STMV-CK; Wognum et al., 1984), which, along with some closely related polyomavirus isolates, in particular foetal rhesus monkey kidney virus (Parry et al., 1983a), HD virus (Waldeck & Sauer, 1977) and STMV (Rangan et al., 1974), was originally isolated from monkey kidney cell cultures derived from several monkey species (reviewed by Schuurman et al., 1991). After the initial isolation of these viruses, it was assumed that the respective monkey species from which the kidney cells originated were the natural hosts of these antigenically related viruses. However, antibodies against these viruses could not be detected in any of the monkey species. In contrast, a high prevalence of antibodies directed against BP\(\text{y}^+\)V was observed in cattle (Parry et al., 1983b; Wognum et al., 1984). Additionally, viruses antigenically related to the virus isolates mentioned above were isolated from bovine kidney cell cultures (Coackley, 1980; Westcott et al., 1987). These data strongly suggest that all the viruses mentioned are of bovine origin and probably entered the cell cultures as contaminants of the calf serum used to supplement the tissue culture medium.

The use of bovine serum, obtained from either foetal or newborn calves, is widespread in biological and medical laboratories. Commercial batches of calf serum usually consist of the pooled sera collected from more than 100 animals. Since the prevalence of antibodies against BP\(\text{y}^+\)V is very high in cattle and polyomaviruses are generally assumed to infect their natural host persistently, the presence of BP\(\text{y}^+\)V in a high percentage of commercial batches of calf serum used for cell culture is likely. This indicates that there is a serious risk of BP\(\text{y}^+\)V contamination of cell cultures used for research, diagnostic or production purposes.

To be able to detect the virus in calf serum as well as in cell cultures and biological reagents, we developed a highly sensitive assay for the detection of BP\(\text{y}^+\)V using the polymerase chain reaction (PCR; Saiki et al., 1988). The sensitivity and specificity of the assay were subsequently evaluated using a series of 20 calf serum batches obtained from nine different suppliers. The experiments were performed without prior knowledge of the biological status of the sera with respect to BP\(\text{y}^+\)V (antibodies or virus isolation). The PCR experiments resulted in the detection of BP\(\text{y}^+\)V DNA in as many as 70% of the calf serum batches. These results were subsequently compared to the presence of antibodies in the sera. In addition, results obtained using the PCR assay were confirmed by virus isolation.
Methods

Bovine sera. Samples of 16 batches of foetal calf serum (FCS), three batches of newborn calf serum (NCS), one batch of adult bovine serum (ABS) and one batch of foetal equine serum (FES) were obtained from nine different commercial suppliers (Bocknek, Boehringer, Brunschwig Chemie, Flow, Gibco, HyClone, Imperial Laboratories, Sanbio and Seromed). The sera were stored at -20 °C on arrival at the DNA extraction laboratory. This laboratory was physically separated from the PCR analysis/recombinant DNA laboratory.

Nucleic acid extraction from serum or PBS. Nucleic acid isolations from the bovine sera or, as a negative control, from PBS were performed at the DNA extraction laboratory, essentially according to the method described by Boom et al. (1990), using the chaotropic agent guanidinium isothiocyanate (GuSCN) and silica particles.

Bovine serum or PBS (1 ml) was added to a 15 ml polypropylene tube (Falcon) containing 9 ml of the GuSCN lysis mixture (L6) and 40 μl of a suspension containing silica particles (SC). The silica particles bind nucleic acid in the presence of high concentrations of GuSCN. The mixture was incubated for 10 min at room temperature and, subsequently, the tubes were spun at 600 g for 5 min to pellet the silica particles. The supernatant was removed by suction using disposable pipettes and the silica pellet was suspended in 1 ml of GuSCN washing solution (L2) prior to being transferred into a 1.5 ml Eppendorf tube. The extraction procedure was completed by washing the nucleic acid-coated silica particles once again with 1 ml L2, twice with 1 ml of 70% ethanol and once with 1 ml of acetone, as described by Boom et al. (1990). Elution of the silica-bound nucleic acid material was performed in 250 μl Tris-EDTA buffer for 30 min at 56 °C using an Eppendorf heat block (model 5320). After vortexing, the suspension was centrifuged for 2 min at 12000 g and the supernatant containing the nucleic acid was transferred into a new vial and stored at -20 °C.

The extraction of nucleic acid material from each serum sample was performed in parallel with a negative control sample containing PBS to monitor possible sample-to-sample transmission of BPyV DNA. These two samples were kept together during the entire extraction and amplification procedure; the serum sample was always handled before the negative control sample. The complete extraction procedure was performed simultaneously for 12 samples (six serum samples and six PBS samples).

Amplification primers. Two primer pairs were selected for the amplification of two different regions of the BPyV genome (Fig. 1); each primer contained 20 to 23 nucleotides complementary to the BPyV genome. An extension of seven to 10 nucleotides at the 5' end of each of these primers contained distinct restriction enzyme recognition sequences to enable cloning of the amplification product into a plasmid vector. The primers were synthesized using an oligonucleotide synthesizer (381A; Applied Biosystems).

The location and exact sequence of the primers are shown in Fig. 1. The primers BPyV-r110 and BPyV-l110, referred to as primer pair A, were used to amplify the sequences between nucleotides 436 and 721, located on the late side of the BPyV genome. This resulted in an amplification product of 306 nucleotides, including the restriction enzyme recognition sequences incorporated at the 5' end of the primer sequences. The second primer pair, BPyV-r150 and BPyV-l150, referred to as primer pair B, was used to amplify the sequences between nucleotides 2744 and 3130, located at the 3' end of the large T coding sequence. This resulted in an amplification product of 400 nucleotides, including the linker sequences present at the 5' ends of both primers.

Amplification procedure. To protect the PCR reaction mixtures and stock solutions from contamination with BPyV (DNA) molecules, the preparation and storage facilities of these solutions were physically separated from both the DNA extraction and PCR analysis laboratories.

The amplification reaction (in 500 μl vials) was performed in a 50 μl volume and the samples were covered with paraffin oil. Each sample was amplified using both primer pairs A and B in separate amplification reactions. The reaction mixture consisted of 50 mM-Tris-HCl pH 8.3, 25 mM-KCl, 100 μg/ml bovine serum albumin (molecular biology grade; Boehringer), 200 μM of each dNTP (Pharmacia), 100 ng of each of the amplification primers and 1 unit of AmpliTaq DNA polymerase (Cetus Corporation). In addition, MgCl₂ was added to a final concentration of 2.5 mM for primer pair A and 3.5 mM for primer pair B. Each amplification reaction was performed on 10% of the extracted nucleic acid material obtained from 1 ml of serum or PBS. In parallel to the amplification of these samples, positive control reactions were performed on all the samples to check each of the reaction mixtures for its amplification potential. In addition to 10% of the extracted nucleic acid material, the positive control reactions contained 1000 molecules of purified BPyV DNA.

Amplification reactions were performed using a DNA Thermal cycler (Perkin-Elmer Cetus) according to the following procedure. After 5 min of denaturation at 95 °C, 35 amplification cycles were performed (1 min 95 °C, 1 min 55 °C, 2 min 72 °C), followed by a final incubation at 72 °C for 8 min.

Electrophoresis and blotting. The amplified material (15 μl) was analysed on a 2% agarose gel using the Tris-acetate-EDTA buffer system (Maniatis et al., 1982), after which the DNA was transferred to nylon membrane filters (Zeta-Probe; Bio-Rad) using the alkaline blotting procedure recommended by the manufacturer. In slot blot experiments, the DNA was transferred to nitrocellulose membranes (BAS 85; Schleicher & Schuell).

Hybridization. Hybridization experiments were performed using an [α-32P]dCTP-labelled probe of the complete BPyV genome. Labelling of the probe with [α-32P]dCTP (specific activity 800 Ci/mmol; NEN) was done using a kit for random-primed labelling (Boehringer).

Prior to hybridization, Zeta-Probe and nitrocellulose filters were incubated for 2 h at 65 °C in prehybridization buffer (3 × SSC, 10 ×...
Denhardt’s solution, 50 μg/ml denatured salmon sperm DNA and 0.1% SDS. Overnight hybridization was performed at 65°C in 6× SSC, 50 μg/ml denatured salmon sperm DNA, 0.1% SDS and 10% dextran sulphate. Filters were washed five times for 30 min each in 1× SSC, 0.1% SDS, and then twice for 20 min each in 0.1× SSC, 0.1% SDS, all at 65°C. Autoradiography was performed using Fuji-RX films.

**Virus isolation.** To concentrate the virus particles present in the sera as well as to clear them from uncomplexed immunoglobulin molecules, 10 ml of serum was ultracentrifuged (3 h, 75000 g in a Beckman SW41 rotor). The pellet was suspended in 250 μl of PBS and subsequently added to 6 ml of Eagle-Earle medium supplemented with 2% Ultroser-G (Gibco) instead of calf serum. Subconfluent *Macaca fascicularis* kidney cell cultures in 60 mm Petri dishes were subsequently incubated with 3 ml of these medium mixtures. After 2 days of incubation, the suspension was changed for normal growth medium supplemented with 2% Ultroser-G. Mock cell cultures were incubated continuously in medium containing 2% Ultroser-G. Cell cultures were examined over the following 8 weeks for the development of BPyV-associated cytopathology.

*M. fascicularis* cells used in these experiments were obtained from the National Institute for Public Health and Environmental Protection, Bilthoven, The Netherlands. The cells had previously been cultured for three passages in tissue culture medium containing FCS. The sera used had been screened for BPyV by virus isolation and immunofluorescence prior to being used for culturing of the primary monkey kidney cells.

**Antibody detection assay.** All serum batches used for the detection of BPyV DNA by means of PCR were also tested for the presence of antibodies against the virus.

The presence of IgG antibodies in the sera was investigated by an indirect immunofluorescence assay, using a fluorescein-conjugated rabbit anti-cow second antibody (Nordic). Kidney cell cultures derived from *M. fascicularis* infected with BPyV were used for antigen presentation. The bovine sera were tested for the presence of antibodies directed against BPyV at a 1:4 dilution.

## Results

**Detection of BPyV DNA in serum**

We developed a sensitive assay for the detection of BPyV DNA using PCR. This assay potentially can be used for the detection of BPyV DNA in cell cultures, vaccines produced *in vitro*, biological reagents and sera. The usefulness of the detection system was evaluated on a coded set of 21 commercial serum batches. To reduce the chance of obtaining false negative results due to intratypic sequence variation, all samples were amplified using two primer sets.

As shown in Table 1, BPyV DNA was detectable with at least one primer pair in 14 of 20 bovine serum batches tested. In 12 of these ‘PCR-positive’ sera, BPyV-specific sequences were detected by both primer sets. No BPyV DNA could be detected in the single batch of FES tested.

The results of an amplification experiment performed on the nucleic acid material isolated from eight different sera (R numbers) and corresponding PBS samples (C) are shown in Fig. 2. BPyV DNA could be detected in four of these sera, whereas all negative control samples remained negative throughout the amplification procedure (Fig. 2, column A, control samples). As shown, the signal intensity of the amplified material in the BPyV-positive sera varied significantly, most probably due to differences in the amount of BPyV DNA present in each of the samples. To a lesser extent, variation in signal intensity might also have been introduced by minor

![Fig. 2. Results of a typical PCR experiment using primer pair A. An amplification reaction mixture (5 μl) was slot-blotted and hybridized using a 32p-labelled BPyV probe. Column A, results of PCR reactions performed on either DNA extracted from 100 μl serum (R numbers) or PBS (C); column B, positive control PCR reactions containing, in addition to the DNA extracted from the serum or PBS samples, 1000 molecules of BPyV DNA as input in the PCR reaction. Vertical bars indicate serum and PBS samples that were extracted and amplified pairwise to monitor cross-contamination of the DNA extracted from the sera. Autoradiography was performed for 18 h at -70°C.](image)

![Table 1. Detection of BPyV, antibodies and viral DNA in serum batches](table)

<table>
<thead>
<tr>
<th>Serum source</th>
<th>Antibody detection</th>
<th>PCR primer A</th>
<th>PCR primer B</th>
<th>Virus isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td>R3</td>
<td>FCS</td>
<td>+</td>
<td>+</td>
<td>NT*</td>
</tr>
<tr>
<td>R7</td>
<td>FCS</td>
<td>+</td>
<td>+</td>
<td>NT</td>
</tr>
<tr>
<td>R9</td>
<td>ABS</td>
<td>+</td>
<td>+</td>
<td>NT</td>
</tr>
<tr>
<td>R10</td>
<td>FCS</td>
<td>+</td>
<td>-</td>
<td>NT</td>
</tr>
<tr>
<td>R13</td>
<td>FCS</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>R15</td>
<td>FCS</td>
<td>+</td>
<td>+</td>
<td>NT</td>
</tr>
<tr>
<td>R18</td>
<td>NCS</td>
<td>+</td>
<td>+</td>
<td>NT</td>
</tr>
<tr>
<td>R19</td>
<td>NCS</td>
<td>+</td>
<td>+</td>
<td>NT</td>
</tr>
<tr>
<td>R21</td>
<td>FCS</td>
<td>+</td>
<td>-</td>
<td>NT</td>
</tr>
<tr>
<td>R22</td>
<td>FCS</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R23</td>
<td>FES</td>
<td>-</td>
<td>-</td>
<td>NT</td>
</tr>
<tr>
<td>R24</td>
<td>FCS</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>R25</td>
<td>FCS</td>
<td>-</td>
<td>+</td>
<td>NT</td>
</tr>
<tr>
<td>R26</td>
<td>FCS</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R27</td>
<td>FCS</td>
<td>+</td>
<td>-</td>
<td>NT</td>
</tr>
<tr>
<td>R28</td>
<td>FCS</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R29</td>
<td>FCS</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R30</td>
<td>FCS</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>R31</td>
<td>FCS</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>R32</td>
<td>FCS</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R33</td>
<td>NCS</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* NT, Not tested.
Fig. 3. Comparison of the sensitivity of the PCR assays performed with primer pair A (A) and primer pair B (B). The number of purified BPyV DNA molecules used as input in each amplification reaction is indicated. Samples were diluted in Tris-EDTA buffer. Zero molecules as input in a reaction indicates that dilution buffer was used as input. The samples were amplified for 35 cycles in a 50 μl volume. Subsequently, 10% of the amplification products were slot-blotted and hybridized as described. Autoradiography was performed for 18 h at -70°C.

differences in the amplification efficiency of separate reactions. This is illustrated by comparing the signal intensity of each of the positive control reactions performed on the extracted PBS samples.

The absolute sensitivity of the PCR assay was investigated by amplification of sequential dilutions of the BPyV DNA molecules used as input in the reaction. After amplification of the DNA and analysis of the amplified material by subsequent hybridization, it was demonstrated that the lower limit for detection was less than 10 molecules of BPyV DNA for both primer sets, although the sensitivity appeared to be slightly greater with primer pair A than with primer pair B (Fig. 3).

Detection of anti-BPyV antibodies in serum

Prior to the development of the PCR assay for the detection of BPyV, we routinely used an indirect immunofluorescence assay for the detection of antibodies against BPyV in bovine serum batches. This assay was also performed on the serum batches used here (Table 1). A serum batch was considered antibody-negative when no specific nuclear fluorescence was observed in BPyV-infected monkey kidney cells using a 1:4 dilution of the serum under investigation.

The results of the antibody detection tests (performed by B. van Steenis at the National Institute for Public Health and Environmental Protection) remained coded until the PCR experiments were completed. As demonstrated in Table 1, antibodies against BPyV could be detected in seven of the calf serum batches tested, whereas antibodies were not detectable in 11 serum batches. The anti-BPyV antibody status was equivocal in the three NCS pools.

Comparison of both detection systems

Antibodies against BPyV could be detected in only five of 14 PCR-positive sera, as demonstrated by comparison of the results obtained using the PCR assay with those obtained using the antibody detection system (Table 1). In another three PCR-positive sera the presence of antibodies was equivocal.

The results obtained with antibody-positive sera R10 and R22 demonstrated that the detection of antibodies directed against BPyV is not always indicative of the presence of viral DNA in the serum.

Virus isolation

The biological relevance of the detection of BPyV DNA in the sera was studied in a virus isolation experiment. In this experiment a set of five PCR-positive and five PCR-negative sera, either containing or lacking anti-BPyV antibodies, was tested. After 4 to 7 weeks of culture, cytopathological changes were observed in all five cell cultures inoculated with PCR-positive serum (Table 1). No BPyV-associated cytopathological changes were observed in mock-infected cell cultures, nor were they observed 8 weeks after inoculation in any of the cell cultures inoculated with PCR-negative serum concentrates (Table 1). To prove that the cytopathological changes observed were the result of a BPyV infection, frozen and thawed tissue culture fluids of each of the cultures were tested for the presence of BPyV by DNA amplification using PCR. To this end, 25 amplification cycles were performed on 5 μl of the tissue culture fluids of all cell cultures using primer pair A. The amplification reactions were performed without prior purification of the nucleic acid material present in the culture fluids. The results of these experiments (Fig. 4) indicate that BPyV DNA could be detected in all cultures inoculated with PCR-positive serum concentrates but not in cultures inoculated with PCR-negative serum concentrates.

It is worth mentioning that BPyV was also isolated from cell cultures inoculated with the R30 serum. As shown in Table 1, BPyV-specific sequences could be detected in this serum only when primer pair B was used. In contrast, after 7 weeks of culture, BPyV-specific sequences could be detected in R30-inoculated monkey
Detection of bovine polyomavirus

Fig. 4. Results of amplification reactions performed on 5 µl frozen and thawed lysates obtained from monkey kidney cell cultures which were either mock-infected (a), or inoculated with concentrated sera that had been shown to be free of BPV by PCR (b) or with concentrated sera containing BPV (c). Samples were amplified for 25 cycles using primer pair A. Subsequently, 10% of the amplified material was analysed on ethidium bromide-stained 2% agarose gels. Ab+ or Ab− indicates whether antibodies against BPV were detectable in each of the sera tested (as also shown in Table 1). (b) Lane 1, R22 (Ab+); lane 2, R26 (Ab−); lane 3, R28 (Ab−); lane 4, R29 (Ab−); lane 5, R32 (Ab−). (c) Lane 1, R13 (Ab+); lane 2, R24 (Ab−); lane 3, R30 (Ab−); lane 4, R31 (Ab−); lane 5, R33 (Ab−). Lanes 6, 123 bp ladder (Gibco).

kidney cell cultures using both primer pairs. Therefore, we concluded that the negative result obtained with primer pair A (Table 1) was not the result of intratypic sequence variation.

The genome of the virus isolated from the cell cultures inoculated with the concentrated R13 serum batch was further characterized by restriction enzyme treatment, followed by Southern blot analysis and hybridization (data not shown). The genome of this virus isolate was shown to be indistinguishable from that of the BPV isolate reported previously (Schuurman et al., 1990). From all these experiments we concluded that the results obtained with the PCR assay constitute a sound basis for predicting the presence of infectious virus in these serum batches.

Discussion

We have used the PCR technique to investigate the presence of BPV DNA in a set of 21 serum batches of bovine or equine origin. The sera, obtained from nine different suppliers, were intended for tissue culture use. Screening of these sera with a BPV-specific PCR assay demonstrated the presence of BPV DNA in 14 of 20 bovine serum batches, suggesting that BPV is a frequent contaminant of calf serum. Positive results were demonstrated in one or more serum batches obtained from each supplier, apart from Bocknek, the supplier of FES.

As illustrated in Fig. 2, the signal intensity obtained after the amplification of BPV-specific sequences was highly variable. Although the PCR assay performed was not a quantitative assay, differences in the amount of amplification product may be a reflection of the amount of virus present in each serum batch.

The lower limit of detection of BPV DNA using the PCR assay was demonstrated to be less than 10 molecules when purified DNA was added directly into the amplification reaction. As shown by Boom et al. (1990), the recovery of DNA using the GuSCN/SC isolation procedure is about 40% when picogram to microgram quantities of DNA are used as input. However, the recovery of DNA when sub-picogram quantities are used is not known. Therefore, we can not estimate the sensitivity of the BPV PCR assay using DNA extracted from serum as input in the amplification reactions.

From the data presented, we conclude that there is no correlation between the detection of antibodies in the serum batches and the detection of BPV DNA. BPV DNA could be detected in 14 of the sera, whereas only five of these were positive by antibody detection. Two additional serum batches (R10 and R22) did contain antibodies against BPV, whereas no BPV DNA could be detected; virus could not be isolated from monkey kidney cell cultures inoculated with one of these sera (R22).

The presence of antibodies against BPV in six serum batches obtained from foetal calves indicates that one or more of the animals had undergone intra-uterine infection because transplacental antibody transmission does not occur in cattle (Butler, 1983).

As already mentioned, the biological relevance of the results of the PCR assay is demonstrated by the isolation of BPV from all five PCR-positive sera. In contrast, no virus was isolated from any of five PCR-negative sera. These results demonstrate that the detection of BPV DNA by PCR correlates well with the isolation of infectious virus from the sera. Therefore, the presence of BPV-specific DNA sequences in bovine products used
for research, diagnostic or production purposes should be a matter of concern. In this respect it is worth mentioning that BPyV belongs to the Polyomaviridae, many of which have been shown to be capable of inducing morphological transformation of cell cultures or inducing tumours in rodents (Salzman, 1986). As recently demonstrated, the viral antigens involved in cell transformation contain an amino acid motif (DLXCXE) that is highly conserved among the DNA tumour viruses (Moran, 1988; Figge et al., 1988). This motif has recently been shown to be essential to the transformation of rodent cells (Chen & Paucha, 1990; Barbosa et al., 1990) and to be involved in the binding of at least one tumour suppressor gene product (RB; Dyson et al., 1989; Ewen et al., 1989; Whyte et al., 1988; DeCaprio, 1988).

The consensus sequence mentioned above is also present in the large T antigen sequence of BPyV (Schuurman et al., 1990). The transforming and tumorigenic capacities of BPyV are under investigation. Preliminary results show that the early region of BPyV is able to transform primary rodent cells into rapidly growing cells capable of anchorage-independent growth and induction of tumours in immunocompromised rats (unpublished results).

From the data presented in this report, it can be concluded that BPyV is a frequent contaminant of calf serum batches intended for cell culture use. To reduce the risk of contaminating cell cultures with BPyV, calf serum batches should be screened for BPyV prior to use for cell culture.

One of the possible strategies to reduce the risk of contaminating serum batches with BPyV is to apply the PCR assay to the screening of individual cows. Subsequently, BPyV-negative herds could be used to breed a population of BPyV-free donor herds. This strategy may make it unnecessary to screen the serum of each individual donor foetus prior to pooling. Another strategy might be to filter the donor sera using filters with a pore size smaller than the size of the virion (about 45 nm). However, the major disadvantages of this strategy may be the loss of certain important serum factors as well as the rapid clogging of the filters with serum proteins.

Alternatively, reducing the risk of contaminating cell cultures with BPyV by inactivating the virus could be attempted. However, polyomaviruses are relatively resistant to inactivation by heat, pH or chemicals. Heat inactivation of a BPyV stock at 56 °C is inefficient, nor can the virus be inactivated efficiently by either acid or alkaline treatment, and infectivity is not affected by chloroform or ether treatment (Westcott et al., 1987).

Gamma-irradiation of infected calf serum might be a means to decrease the infectivity of the virus. However, gamma-irradiation of a serum batch contaminated with high titres of stock virus only resulted in partial inactivation of viral infectivity (B. van Steenis, unpublished results). The cell growth-stimulating properties of the serum were, however, severely reduced by the irradiation process.

The most straightforward method to reduce the chance of contaminating cell cultures with BPyV would appear to be to avoid the use of calf serum by supplementing the tissue culture medium with a serum substitute. However, one should be aware that even these substitutes do contain some components of bovine origin and may still contain (infectious) BPyV. For this reason, we are evaluating the presence of BPyV DNA in these substitutes.

We would like to thank R. Boom for helpful discussions, and W. Hersbach and W. Munk for technical support. This research was supported by the Technology Foundation (STW).

References


(Received 4 June 1991; Accepted 29 July 1991)