Goat Herpesviruses: Biological and Physicochemical Properties

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SUMMARY

Two herpesvirus isolates from goats are known which cause afflictions of the digestive tract in kids and, in some cases, abortion. An antigenic relationship of these goat herpesviruses with infectious bovine rhinotracheitis/infectious pustular vulvovaginitis virus (bovid herpesvirus 1, BHV-1) was reported and because of the species-specific pathogenicity, the goat isolates were named caprine herpesvirus 1. In this report the two isolates are further characterized and compared with BHV-1. Although the caprine herpesviruses share many biological and physicochemical properties with BHV-1, they can be differentiated from the bovine viruses with respect to growth cycle, one-way cross-neutralization and, most importantly, the restriction endonuclease fragments of their DNAs. The molecular weight of the caprine herpesvirus DNA, based on electron microscopic length measurement is $90 \times 10^6$, similar to that of BHV-1 ($95 \times 10^6$). On the basis of these genomic differences, we propose that DNA restriction endonuclease patterns of the caprine herpesviruses should be designated as prototypic of bovid herpesvirus 6 (BHV-6).

INTRODUCTION

Herpesviruses from goats have only been isolated in California (Saito et al., 1974) and Switzerland (Mettler et al., 1979). The American strain was characterized in some detail (Berrios & McKercher, 1975) and was found to be serologically related to infectious bovine rhinotracheitis/infectious pustular vulvovaginitis (IBR/IPV) virus (bovid herpesvirus 1, BHV-1). Since it was not pathogenic for lambs and calves, it was referred to as caprine herpesvirus 1 (Berrios et al., 1975). The clinical and pathological symptoms caused by the Swiss isolate are similar to those reported for the Californian strain, although abortifacient properties could not be substantiated (Waldvogel et al., 1981). In a sero-epidemiological survey, 20 to 30% of the goats in the eastern and southern parts of Switzerland exhibited specific antibodies, whereas goats examined in other regions seemed to be free of infection (Plebani et al., 1983). In the Bregaglia valley, where the isolation was made and where this herpesvirus infection is endemic in goats, BHV-1 infections have never been observed in cattle. Recent serological studies have shown that goat herpesvirus infections also occur in Greece, Turkey and Syria, indicating widespread latency of the virus (T. Leiskau & H. Ludwig, unpublished results).

Since BHV-1 infections may occur in goats under natural conditions (Mohanty et al., 1972), we were interested to find out whether the goat viruses and BHV-1 were identical. In this report, further biological properties and physicochemical characteristics of the two goat herpesvirus strains are presented. DNA analysis has shown fundamental differences between BHV-1 and the goat virus. In accordance with a proposed classification of bovine (bovid) herpesviruses (Ludwig, 1983), and based on the recommendations of the Committee for Taxonomy of Viruses (Roizman et al., 1981), the goat herpesvirus is designated as bovid herpesvirus 6 (BHV-6).

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**Methods**

**Viruses and cell cultures.** Two goat herpesviruses, E/CH (Engels, Switzerland) and McK/US (McKercher, United States; kindly supplied by Dr D. G. McKercher, Los Angeles, Ca., U.S.A.) were used. The Los Angeles strain of IBR (IBR LA'), IPV K22 (Farley et al., 1981) and the field strain, IPV Sch., served as reference strains for BHV-1. All the viruses were plaque-purified three times. Except where otherwise stated, Georgia bovine kidney (GBK) cells were used for virus growth. They were maintained in Dulbecco’s modified Eagle’s medium (Gibco), containing 100 IU/ml penicillin, 50 μg/ml streptomycin (Serva, Heidelberg, F.R.G.) and 10% inactivated foetal calf serum (Gibco) for growth, or 2% for maintenance.

**Antisera.** Convalescent serum of a goat experimentally infected with strain E/CH herpesvirus (Waldvogel et al., 1981) and various antisera against BHV-1 were kindly supplied by Dr A. Waldvogel, Zürich, and Drs J. P. Gregersen and G. Pauli, Berlin, respectively. Antisera against strain E/CH or BHV-1 (IBR, strain LA, or IPV, strain Sch.) were produced using infected cell lysates. For this purpose 1 × 10^6 infected GBK cells (per injection) were harvested 20 h after infection, pelleted (10 min at 1000 g), suspended in 4 vol. of lysis buffer (0.02 M-glycine, 0.0076 M-Tris-HCl pH 8.6, containing 5% Triton X-100), ultrasonicated for 1 min and centrifuged for 1 h at 35000 rev/min (SW50.1 Beckman rotor). This supernatant containing no infectious material was mixed with Freund’s adjuvant (for procedure and immunization schedule, see below) and used for immunization of seronegative (IBR and E/CH) virus cattle.

To provide a homologous system, BHV-6 or BHV-1, propagated over two passages in primary newborn rabbit brain cells, were used for rabbit inoculation. For each injection dose, 1 × 10^6 cells had been washed with inactivated normal rabbit serum 2 days prior to infection and maintained in medium containing 5% (v/v) of the same serum. The rabbits were inoculated intradermally at different sites at intervals of 10 to 14 days, using lysates mixed 1:1 with Freund’s complete adjuvant for primary inoculation or Freund’s incomplete adjuvant for booster injections. Antisera against bovine herpesvirus (BHV-2), pseudorabies virus, equid herpesvirus 1, canine herpesvirus, herpes simplex virus type 1 (HSV-1), and B virus were standard reference sera of the Institute for Virology, Berlin.

**Growth curves and host range studies.** The determination of extra- and intracellular virus followed standard procedures. GBK monolayers (3.0 × 10^5 cells) were inoculated at a multiplicity of infection (m.o.i.) of 10. For determination of the host range of BHV-6, various primary or secondary cells or cell lines in 6 cm Petri dishes were infected with the E/CH strain using a m.o.i. of 1 for the first passage. For the second and third passages, 0.5 ml of inoculum per dish was used. When 80% cytopathic effect (c.p.e.) was observed the remaining cell sheet was scraped into the medium and frozen at −70 °C. If no c.p.e. was observed, the cells were harvested 7 days after infection. After two cycles of freezing and thawing, and centrifugation (10 min; 10000 g), this supernatant containing no infectious material was mixed with Freund’s adjuvant (for procedure and immunization schedule, see below) and used for immunization of seronegative (IBR and E/CH) virus cattle.

**Virus titrations.** As well as normal 50% endpoint titre determinations in microtitre plates (Falcon 3042), virus assays were performed using standard plaque tests. Briefly, GBK cells were seeded in 24 cluster plates (Nunclon, Nunc) at concentrations of 5.0 × 10^4 cells in 0.2 ml/well; 0.2 ml aliquots from a series of 10-fold virus dilutions were added, using two wells per dilution. After 4 h incubation at 37 °C, 0.4 ml of 1-6% carboxymethylcellulose (BDH) in maintenance medium was added and the plates were further incubated at 37 °C. Three days later the cells were fixed in 4% formalin and stained with Giemsa blood-staining solution.

**Serum neutralization tests.** For plaque reduction tests, sera were diluted with maintenance medium in a series of twofold dilutions, mixed with the same volume of virus suspension containing 1000 p.f.u./ml and incubated for 1 h at 37 °C. A 0.2 ml amount of this mixture was added to 0.2 ml of cell suspension per well. After 4 h at 37 °C, 0.4 ml of 1-6% carboxymethylcellulose in maintenance medium was added per well, and the plates were incubated at 37 °C. The plaque reduction tests were usually read after 3 days. Calculations refer to 80% plaque reduction (Pauli & Ludwig, 1977).

**Crossed immunoelectrophoresis.** The experiments followed essentially the technique reported by Axelsen (1973) and experimental conditions given by Norrild et al. (1978). Briefly, lysates of infected cells were separated in the first dimension and then electrophoresed into the antibody-containing second-dimension gel. Intermediate gels were applied to test antigenic cross-reactivity.

**Buoyant density determinations.** The DNAs were centrifuged isopycnically in caesium chloride following established procedures (Ludwig & Rott, 1975). Briefly, cell cultures infected at a m.o.i. of 10 were supplied with medium containing 10 μCi/ml [3H]thymidine or 1 μCi/ml [14C]thymidine (Bio-Rad) 6 to 8 h after infection. At the end of the labelling time (24 h post-infection), the supernatant virus was centrifuged through a 40% (w/v) sucrose cushion for 1 h at 100000 g (SW41 Beckman rotor) and the pellet was resuspended in TNE buffer (5 mM-Tris-HCl pH 7.4, 100 mM-NaCl, 1 mM-EDTA). Aliquots of 10^6 ct/min of isotopically labelled virus were lysed with SDS and sodium lauryl sarcosinate (LSL) to give a final concentration of 1%, immediately added to CsCl (density, from refractive index, 1.70 g/ml) and centrifuged to equilibrium for 48 h at 39000 rev/min (T150 Beckman rotor). Co-centrifugation of BHV-1 and BHV-6 DNAs, having different labels, was performed to detect minor density differences between the two DNAs (Halliburton et al., 1975). The G + C content of the DNA was calculated from the buoyant density according to Schildkraut et al. (1962).
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DNA extraction and restriction enzyme analysis. For each virus strain 10 Roux bottles with GBK monolayers were infected at a m.o.i. of 5. At 24 h after infection, the cells were scraped off and separated from the supernatant by low-speed centrifugation. The supernatant was centrifuged for 20 min at 10000 rev/min (Sorvall GSA rotor) and then for 1 h at 25000 rev/min (SW27 Beckman rotor). The virus pellet was resuspended in TNE buffer, pelleted again through a 15 ml 30% (w/v in TNE) sucrose cushion for 2 h at 25000 rev/min (SW27 rotor) and resuspended in 1-8 ml TNE buffer to which 200 μl of 10% SDS and 40 μl of proteinase K (10 mg/ml) were added. After overnight incubation at 37°C, DNA was extracted with phenol–chloroform (1 : 1), chloroform–isoamyl alcohol (24 : 1) and precipitated with ethanol. The DNA was redissolved in 10 mM-Tris-HCl pH 7-5, 1 mM-EDTA and stored at 4°C.

Restriction endonuclease digestions with EcoRI, HindIII, BamHI and BstEII (Boehringer Mannheim) were performed under conditions recommended by the supplier. The digestion products were separated by electrophoresis in 0.7% agarose (Sigma) slab gels. The gels were run at 35 V for 18 h, then stained in a bath of 1 μg/ml ethidium bromide in electrophoresis buffer (Tris-acetate buffer: 40 mM-Tris, 2 mM-EDTA, 20 mM-acetic acid) for 30 min and photographed under a u.v. lamp through a red filter using Polaroid Land film type 57 or HP4 Ilford films.

Other methods. The thermal denaturation profile and the T_m value were determined as previously outlined (Darai et al., 1980). Electron microscopic contour length measurements were performed according to Davis et al. (1971).

RESULTS

Cell culture behaviour of BHV-6 and BHV-1

The cytopathic effect of BHV-1 and BHV-6 in bovine cells is characterized by rounding of the cells (Berrios & McKercher, 1975; McKercher, 1964; Mettler et al., 1979). In contrast to BHV-1, however, BHV-6 induced a more diffuse c.p.e. with prominent swelling of cells and vacuole formation, followed by detachment and clump formation (Fig. 1). The growth curve of BHV-6, strain E/CH, is comparable to that reported by Berrios et al. (1975), using the McK/US strain. The viruses have a latent period of 5 h and an exponential growth phase 6 to 12 h after infection. BHV-6 is one of the most rapidly growing herpesviruses known, reaching maximum titres 12 h post-infection almost identical to BHV-1 (Fig. 2).

BHV-6 multiplies to relatively high titres without adaptation in a broad spectrum of cell cultures, similar to BHV-1 (Gibbs & Rweyemamu, 1977). The host range studies which confirm and extend those for the McK/US strain (Berrios & McKercher, 1975) are summarized in Table 1.

Serological relationship of BHV-6 with other herpesviruses

Reference antisera directed against BHV-2 (bovine herpes mammillitis virus), pseudorabies, canine and equid (EHV-1) herpesvirus, HSV-1 and B virus did not neutralize BHV-6 strain E/CH, even at serum dilutions of 1:2.

Because of the known cross-reactivity of BHV-6 and BHV-1, various antisera and virus strains were checked in order to test whether the reaction might be influenced by the species of the antiserum donor or by the virus strain. Independently of these factors, a one-way cross-neutralization could be clearly demonstrated. BHV-1-specific antisera neutralized BHV-6 to a much greater extent than BHV-6-specific antisera did BHV-1 (Table 2). Preliminary results using monoprecipitin antisera (kindly supplied by Dr J. P. Gregersen) directed against the major immunogenic components of BHV-1 confirmed the above finding.

That the antigenic relationship of BHV-6 and BHV-1 extends to the major antigenic components of BHV-6 was demonstrated by crossed immunoelectrophoresis using the intermediate gel technique. Although BHV-6-specific antisera recognized the major antigens of BHV-1, this was to a varying extent, as shown for precipitation arcs 1 and 2 (Fig. 3).

Properties of the BHV-6 DNA

Optimal labelling of BHV-6 DNA was achieved when radioactive isotopes were added 6 to 8 h after infection. Preliminary experiments, labelling infected cells at 2, 4, 6 and 8 h, showed that at 4 h post-infection cellular DNA synthesis was significantly decreased and at 8 h completely shut down. The buoyant density of radioactively labelled virus DNA, determined by
Fig. 1. Cytopathic effect caused by BHV-6 (E/CH) and BHV-1 (IBR 'LA'). (a) Uninfected bovine foetal lung cells; (b) 12 h after infection with E/CH; (c) 24 h after infection with IBR 'LA'; (d) 36 h after infection with E/CH; (e) 48 h after infection with IBR 'LA'. (Magnifications: a, b, d x 560, c, e x 224.) BHV-6-infected cells (b, d) show swelling and vacuolization, followed by detachment in clumps, whereas BHV-1-infected cells (c, e) round off and detach.

Preparative ultracentrifugation, was 1.7295 g/ml. Co-centrifugation of labelled BHV-6 and BHV-1 DNAs confirmed this result, demonstrating identical buoyant densities for the caprine and the bovine viruses (Fig. 4). From these data a G+C content of 71% was calculated.

Melting of BHV-6 DNA in 0.1 x saline sodium citrate (SSC) followed a sigmoidal curve, from which a Tm of 86°C was derived (data not shown). This value corresponds to a G+C content of 72% which is in good agreement with that obtained from buoyant density determinations.

Electron microscopic examinations of BHV-6 and BHV-1 DNAs (n = 19, each), showed linear molecules with an average contour length of 46.1 μm for BHV-1 and 43.7 μm for BHV-6 DNA (Fig. 5 and 6). Using phage PM2 DNA as an internal length marker (kindly supplied by
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Fig. 2. Growth curve of BHV-6 (E/CH, O; intracellular McK/US, □) and BHV-1 (IBR 'LA', □) in GBK cells. Inoculation was done at a m.o.i. of 10. Total or intracellular virus titres were measured. Maximum virus yield was reached 12 h post-infection for BHV-6 and at 20 h for BHV-1 (IBR 'LA'). The titre differences after 2 h post-infection may reflect differences in ratios of physical to infectious particles of the virus stocks or differences in adsorption capacity.

Table 1. Host cell range of BHV-6 strain E/CH and titres reached in different cell systems

<table>
<thead>
<tr>
<th>Species</th>
<th>Cell culture</th>
<th>Titre (log_{10}/ml) at passage no.*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Human</td>
<td>Foetal oligodendroglia</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>HEL (embryonic lung)</td>
<td>5.5</td>
</tr>
<tr>
<td>Monkey</td>
<td>MA 104 (kidney)†</td>
<td>4.25</td>
</tr>
<tr>
<td>Mouse</td>
<td>DBT (brain)</td>
<td>5.0</td>
</tr>
<tr>
<td>Rabbit</td>
<td>JKG (brain)</td>
<td>3.75</td>
</tr>
<tr>
<td>Mink</td>
<td>ML (lung)†</td>
<td>4.5</td>
</tr>
<tr>
<td>Cat</td>
<td>CL (lung)†</td>
<td>4.5</td>
</tr>
<tr>
<td>Dog</td>
<td>MDCK (kidney)†</td>
<td>5.0</td>
</tr>
<tr>
<td>Swine</td>
<td>PK 15†</td>
<td>5.75</td>
</tr>
<tr>
<td>Cattle</td>
<td>BFS (foetal skin)</td>
<td>4.75</td>
</tr>
<tr>
<td></td>
<td>BK (kidney)</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>GBK (kidney)†</td>
<td>6.25</td>
</tr>
<tr>
<td>Chicken</td>
<td>Embryonic fibroblasts</td>
<td>0</td>
</tr>
</tbody>
</table>

* GBK cells were used for all titrations; titres represent total virus yield (see Methods).
† Cell lines.

Dr D. Simon, Berlin), mol. wt. of 90 × 10^6 or 95 × 10^6 were determined for BHV-6 or BHV-1 DNA, respectively (Stüber & Bujard, 1977). These values correspond to 136 or 143 kilobase pairs (kb). Additionally, EcoRI fragments of BHV-6 DNA were eluted from gels and measured electron microscopically. The larger fragments had an average length of 30-46 ± 1.2 μm (n = 17) and the smaller ones 13-64 ± 0.9 μm (n = 26). These values correspond to mol. wt. of 62.8 × 10^6 and 28.1 × 10^6, respectively. The sum of the mol. wt. of EcoRI fragments was 90.9 × 10^6 which is in excellent agreement with the mol. wt. estimated for the intact DNA. The physico-chemical data are summarized in Table 3 and compared with pre-existing values for BHV-1.

Since no significant differences were detected between BHV-1 and BHV-6 DNA, it was of major interest to compare the genomes by restriction enzyme analysis. Both BHV-6 strains showed only minor differences and can therefore be considered as different isolates of the same virus. BHV-6 DNA had only one recognition site each for endonucleases EcoRI and HindIII. To our knowledge, this is the only herpesvirus DNA demonstrating this unique cleavage pattern.
Fig. 3. Crossed immunoelectrophoresis demonstrating reactivity of BHV-6-specific antibodies with major antigens of BHV-1. Infected GBK cell lysates (30 μl) of BHV-1 (IBR 'LA') were electrophoresed in the first dimension (f) at 10 V/cm for 1.5 h, followed by second dimension electrophoresis (2 V/cm for 18 h) into gels (s) containing polyspecific bovine BHV-1 antibodies (15 μl/cm²). The intermediate gels (i) contained 20 μl/cm² of foetal calf serum (A) or BHV-6-specific goat antibodies (B). Uninfected cell lysates gave no precipitation arcs with the same sera (Ludwig, 1983). Coomassie Brilliant Blue staining.

Table 2. **Cross-neutralization of BHV-6 and BHV-1 using cattle and goat antisera***

<table>
<thead>
<tr>
<th>Virus</th>
<th>E/CH (cattle)†</th>
<th>E/CH (goat)‡</th>
<th>IBR 'LA' (cattle)†</th>
<th>IBR (NCS)§</th>
<th>IPV Sch. (cattle)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>E/CH</td>
<td>1:104</td>
<td>1:649</td>
<td>1:20</td>
<td>ND</td>
<td>1:42</td>
</tr>
<tr>
<td>McK/US</td>
<td>1:108</td>
<td>1:605</td>
<td>ND</td>
<td>1:61</td>
<td>1:77</td>
</tr>
<tr>
<td>IBR 'LA'</td>
<td>1:4</td>
<td>1:11</td>
<td>1:28</td>
<td>ND</td>
<td>1:104</td>
</tr>
<tr>
<td>IPV K22</td>
<td>1:8</td>
<td>ND</td>
<td>ND</td>
<td>1:143</td>
<td>1:155</td>
</tr>
<tr>
<td>IPV Sch.</td>
<td>1:7</td>
<td>1:9</td>
<td>ND</td>
<td>1:108</td>
<td>1:145</td>
</tr>
</tbody>
</table>

* Cross-neutralization tests showed that antisera against BHV-1 strongly neutralize BHV-6, whereas antisera against BHV-6 neutralize BHV-1 only weakly. Similar results were obtained when rabbit antisera were used (data not shown). Titres are expressed as the serum dilution neutralizing 80% of the plaques.
† Immunized animals.
‡ Convalescent serum after experimental infection.
§ Newborn calf serum, used for tissue culture.
ND, Not done.

The restriction fragments of BHV-6 DNA differed completely from that of BHV-1 DNA (Fig. 7). The mol. wt. estimated by summation of mol. wt. of single fragments, using lambda DNA as the size marker, agreed well with calculations based on molecule length (Table 3).
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Fig. 4. Buoyant density determination of BHV-6 and BHV-1 DNAs by preparative ultracentrifugation in caesium chloride. Co-centrifugation experiments of BHV-1 (IBR 'LA') DNA labelled with $^{3}$H]thymidine (C) and BHV-6 (E/CH) DNA labelled with $^{14}$C]thymidine (●) showed that the DNAs had the same buoyant density (....).

DISCUSSION

The purpose of this report was to characterize the two established caprine herpesvirus isolates and differentiate them from BHV-1. Certain properties indicated that the goat virus might represent an independent herpesvirus (Berrios et al., 1975). The susceptibility of goats to BHV-1 and the occurrence of this virus in goats, however, made differentiation between the two viruses difficult. Our data have shown that the goat virus, BHV-6, and BHV-1 can be distinguished by c.p.e., by the more rapid growth cycle of BHV-6, and serologically by a clearly defined one-way neutralization. Since a strong antigenic cross-reactivity exists, it will be of interest to define common sites on these antigens.

The restriction endonuclease analysis of the viral DNAs clearly demonstrates that the caprine and bovine viruses are distinct and confirms their designation as BHV-6 and BHV-1, although significant differences in physicochemical properties of their DNAs do not exist. This technique has served as a valuable tool for separation and classification of numerous species, types and strains of herpesviruses, as previously reported for bovid (Engels et al., 1981; Ludwig, 1983), equid (Studdert et al., 1981), suid (S. Herrmann, B. Heppner & H. Ludwig, unpublished results) and human herpesviruses (Preston et al., 1978; Buchman et al., 1978).

The two bovid herpesviruses, which appear to be indigenous in different animal species of the family Bovidae, BHV-6 in goats and BHV-1 in cattle, have either maintained or developed a strong antigenic relationship during evolution, but are completely divergent with respect to their DNA restriction endonuclease maps. Surprisingly, these two viruses have been reported to be immunologically distinct (Berrios et al., 1975), although preliminary data indicate that considerable cross-reactivity exists between their major immunogenic components (J. P. Gregersen, personal communication). Thus, it will be of great interest to elucidate whether this closely related pair of viruses is farther apart in the evolutionary sense than herpes simplex virus types 1 and 2. According to the concept of unity and diversity of herpesviruses (Honess &
Fig. 5. Electron microscopic demonstration of BHV-6 (E/CH) DNA. The micrograph shows that BHV-6 DNA is a linear double-stranded molecule (arrow). PM2 DNA (star shape) was used as an internal standard length marker.
Fig. 6. Length determination of BHV-6 (E/CH, ) and BHV-1 (IBR ‘LA’, ) DNA by electron microscopy (n = 19). BHV-6 and BHV-1 DNAs had average contour lengths of 43.7 ± 0.9 μm and 45.9 ± 1.5 μm respectively. Taking into consideration the standard deviations, the difference is not significant.

Fig. 7. Restriction enzyme analysis of BHV-6 and BHV-1 DNAs. Lambda DNA served as molecular weight marker (lanes, 1, 8 and 12). Digestions with EcoRI: IBR ‘LA’, E/CH, McK/US (lanes 2, 3 and 4); digestions with HindIII: IBR ‘LA’, E/CH, McK/US (lanes 5, 6 and 7); digestions with BstEII: IBR ‘LA’, E/CH, McK/US (lanes 9, 10 and 11); E/CH digested with BamHI, BamHI + BstEII, BstEII (lanes 13, 14 and 15); McK/US digested with BamHI, BamHI + BstEII, BstEII (lanes 16, 17 and 18). The restriction patterns show that the two BHV-6 strains are closely related but different from BHV-1.

Table 3. Physicochemical properties of BHV-6 and BHV-1 DNA

<table>
<thead>
<tr>
<th></th>
<th>BHV-6</th>
<th>BHV-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buoyant density in CsCl (g/ml)</td>
<td>1.7295</td>
<td>1.7295 (1.730; Ludwig, 1972)</td>
</tr>
<tr>
<td>G + C (%)</td>
<td>71</td>
<td>71 (71.5; Ludwig, 1972)</td>
</tr>
<tr>
<td>Tm (0.1 × SSC) (°C)</td>
<td>86</td>
<td>85.5 (Ludwig, 1972)</td>
</tr>
<tr>
<td>G + C (%)</td>
<td>72</td>
<td>71.5 (Ludwig, 1972)</td>
</tr>
<tr>
<td>Molecule length (μm)</td>
<td>43.7 ± 0.9</td>
<td>45.9 ± 1.5</td>
</tr>
<tr>
<td>Molecular weight* (a)</td>
<td>90 × 10⁶</td>
<td>89.6 × 10⁶ (IBR ‘LA’; Engels et al., 1981)</td>
</tr>
<tr>
<td>(b)</td>
<td>90.1(±1.8) × 10⁶</td>
<td>94.8(±3.1) × 10⁶ (IPV K22; Farley et al., 1981)</td>
</tr>
<tr>
<td>(kb)</td>
<td>135.7 ± 2.7</td>
<td>142.7 ± 4.65</td>
</tr>
</tbody>
</table>

* (a) Calculated from restriction enzyme analysis; (b) calculated from contour length of the molecule.
Watson, 1977), the goat and bovine viruses may reflect the situation with HSV and B virus. Like BHV-6 and BHV-1, the case of HSV and B virus is another example of immunologically related (Vizoso, 1975), but genetically different (Ludwig et al., 1983) herpesviruses.

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