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Genome of Bovine Herpesvirus 5

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Here we present the complete genomic sequence of bovine herpesvirus 5 (BHV-5), an alphaherpesvirus responsible for fatal meningoencephalitis in cattle. The 138,390-bp genome encodes 70 putative proteins and resembles the α2 subgroup of herpesviruses in genomic organization and gene content. BHV-5 is very similar to BHV-1, the etiological agent of infectious bovine rhinotracheitis, as reflected by the high level of amino acid identity in their protein repertoires (average, 82%). The highest similarity to BHV-1 products (≥95% amino acid identity) is found in proteins involved in viral DNA replication and processing (UL5, UL15, UL29, and UL39) and in virion proteins (UL14, UL19, UL48, and US6). Among the least conserved (≤75%) are the homologues of immediate-early (IE) proteins BICP0, BICP4, and BICP22, the three proteins being longer in BHV-5 than in BHV-1. The structure of the BHV-5 latency-related (LR) region departs markedly from that of BHV-1 in both coding and transcriptional regulatory regions. Given the potential significance of IE genes and the LR region in virus-neuron interactions, it is likely these differences contribute to BHV-5 neuropathogenicity.

Bovine herpesvirus 5 (BHV-5) is a pathogen of cattle responsible for sporadic epizootics of fatal meningoencephalitis (6, 37). Due to similarities in virion morphology, cytopathic effects in cell culture, and antigenic properties (37, 38), BHV-5 was formerly regarded as a neuropathogenic variant of bovine herpesvirus 1 (BHV-1), the etiological agent of infectious bovine rhinotracheitis and vulvovaginitis. Subsequent comparative studies based on restriction site mapping of viral DNA (28, 32, 93), cross-neutralization tests, and monoclonal antibody reactivity (24, 63) indicated that the viruses differ in genomic and antigenic properties. In 1992 BHV-5 was recognized as a distinct virus by the International Committee on Taxonomy of Viruses (78).

Both BHV-5 and BHV-1 are neurotropic viruses, but only BHV-5 is capable of significant replication in the central nervous system (CNS) and induction of neurological disease (5, 10). Outbreaks of meningoencephalitis caused by BHV-5 have been reported in Australia (37), North and South America (8, 15, 42, 77), and Europe (9, 66). The course of the disease after experimental infection with BHV-5 depends on the virus isolate, the route of inoculation, and the immunological status and age of the animal. Calves up to 4 months of age are most susceptible. During the first week following intranasal inoculation of virus, the animals either present signs of mild rhinitis and conjunctivitis or they remain asymptomatic (5, 16, 66, 73). At this point, animals can recover from infection or, alternatively, progress to neurological disease and die. Regardless of the appearance of clinical neurological disease in infected calves, BHV-5 invades the CNS and causes various degrees of pathology (16, 65). The neural route followed by BHV-5 to invade the CNS in cattle has not been defined, although both the trigeminal and the olfactory pathways have been implicated (6, 65). In a rabbit model for BHV-5, the olfactory pathway is the main route for neural dissemination (19, 57).

At times when no infectious virus can be isolated from peripheral sites, surviving animals exhibit BHV-5 viral sequences in their trigeminal ganglia (TG), indicating that, as in BHV-1, BHV-5 remains latent in the TG (5, 16, 65, 91). Latent virus can be reactivated after treatment of latently infected animals with dexamethasone (10, 16). Although recrudescence of clinical disease has been observed after experimental virus reactivation, its occurrence in nature remains unknown (73). Previous vaccination of cattle against BHV-1 resulted in protection against BHV-5-induced neuropathology and clinical signs of disease (16). This is partially explained by the cross-reactivity of induced BHV-1 neutralizing antibodies (24). Vaccination with BHV-1, however, does not prevent establishment of latency by BHV-5 (16).

The marked neuroinvasiveness (and often neurovirulence) displayed by BHV-5 contrasts with the inability of BHV-1 to invade the CNS and cause neurological disease to any significant degree. During infection with other neurotropic herpesviruses (e.g., pseudorabies virus [PRV] and herpes simplex virus [HSV]), neuroinvasiveness and local virus spread in the CNS are properties which largely rely on certain viral envelope glycoproteins (46, 67). However, other viral functions could contribute to a successful infection of the CNS.

Comparative genomics has proven useful in identifying genes involved in virulence. The complete BHV-1 genomic sequence, a composite including sequences of five different virus strains, is available (GenBank accession number AJ004801). However, less than 15% of the BHV-5 genome (mostly representing envelope glycoproteins) has been sequenced (1, 18, 20, 22, 31, 40, 64, 80, 86). Here we present the complete sequence of a neurovirulent strain of BHV-5, with analysis and comparison to BHV-1.

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MATERIALS AND METHODS

DNA isolation, cloning, and sequencing. BHV-5 strain SV507/99 was originally isolated from bovine brain tissue from a case of fatal encephalitis in southern Brazil (91).

To obtain viral DNA, BHV-5-infected MDBK cells were pelleted, washed in NTE buffer (10 mM Tris [pH 8.0], 150 mM NaCl, 5 mM EDTA), resuspended in KTE buffer (10 mM Tris [pH 8.0], 10 mM KCl, 5 mM EDTA), and incubated in KTE buffer containing 0.25% β-mercaptoethanol and 10% Triton X-100 for 10 min on ice. After centrifugation at 3,000 rpm (Sorvall), 5 min, 4°C, virus in the supernatant was pelleted onto a 30% sucrose cushion (13,000 rpm, 1 h, 4°C), resuspended in Tris-EDTA (TE; pH 8.0), incubated in the presence of sodium dodecyl sulfate (0.5% [wt/vol]) and proteinase K (10 mg/ml) for 2 h at 37°C, and phenol extracted. Viral DNA was precipitated with ethanol and resuspended in TE buffer (pH 8.0). Random 1- to 8-kbp DNA fragments were obtained by incomplete enzymatic digestion with *Aci*I endonuclease (New England Biolabs, Beverly, Mass.). DNA fragments of 1.5 to 3 kbp were isolated after separation in size exclusion columns (Clontech), cloned into the dephosphorylated *Aci*I site of pUC19 plasmids, and grown in *Escherichia coli* DH10B cells (Gibco BRL, Gaithersburg, Md.). Plasmids were purified by alkaline lysis as instructed by the manufacturer (Eppendorf 5 Prime; Boulder, Colo.). DNA templates were sequenced from both ends with M13 forward and reverse primers and from selected plasmids with transposon insertion (EZ::TN<KAN-2> transposon insertion kit; Epicentre Tech., Madison, Wis.), using dideoxy-chain terminator sequencing chemistries (82) and an Applied Biosystems PRISM 3700 automated DNA sequencer (PE Biosystems, Foster City, Calif.). Bases were called from chromatogram traces with Phred (35), which also produced a quality file containing a predicted probability error at each base position.

DNA sequence analysis. DNA sequences were assembled with Phrap (34), using the quality files and default settings to produce a consensus sequence which was manually edited with Consed (41). An identical sequence was assembled using the Cap3 assembler with quality files and clone length constraints (45). The final DNA consensus sequence represented an average eightfold redundancy at each base position. Gap closure was achieved by primer walking of gap-spanning clones and sequencing of PCR products. A total of 7,835 usable traces were assembled into a 127,162-bp contig by bidirectional sequencing of random clones and 61 PCR products, including those that crossed the terminal junction sequences from concatemeric replicative intermediates. The assembled contig had an estimated error rate of <0.03/10 kbp and showed no evidence of polymorphism using Polyphred analysis (34), including within the single, complete unique short (U_S) repeat sequence which assembled with double average redundancy at each base position, consistent with a bimolar representation of repeat sequences derived from two identical copies, as found in other alphaherpesviruses. Thus, the assembled contig contained 570 bp of the terminal repeat (TR) at the left terminus, all of the unique long (U_L), internal repeat (IR), and U_S sequences, and 738 bp of the TR at the right terminus. IR and TR sequences were also assembled separately with clones containing the unique-repeat junctions and overlapping clones, using length constraints and position as provided by the computer assembly programs. These assemblies were manually joined at the unique-repeat boundaries, thus providing the complete genome. For descriptive purposes, we have presented BHV-5 in a linearized fashion as described by Dolan et al. (29). Genome DNA composition, structure, repeats, and restriction enzyme patterns were analyzed as previously described (2). Open reading frames (ORFs) encoding proteins of ≥60 amino acids with a methionine start codon (88) were evaluated for coding potential using the Hexamer (ftp.sanger.ac.uk/pub/rd) and Glimmer (81) computer programs. Other criteria included similarity to other herpesvirus and compact gene arrangements with little gene overlap. Homology searches were conducted using BLAST (3), PSIBLAST (4), FASTA (72), BLIMPS (92), and HMMER (87) programs with the Prosite, Pfam, Prodom, Sbase, Blocks, Domo, and GenBank databases (14). GCG (26), MEMSAT (54), and SAPS (12) programs were used for gene analysis. The coding potential and splicing patterns in the latency-related (LR) region of BHV-5 and BHV-1 were analyzed with Glimmer, Hexamer, Splice (a neural network program for eukaryotic splice site prediction; ftp://genome.lbl.gov/pub/reese/SPLICE), NNPP (eukaryotic promoter prediction; ftp://genome.lbl.gov/pub/reese/NNPP), TIGR GeneSplicer (74), HMM gene trained on human and *Drosophila* gene sets (55), and Eponine for transcription start site detection (30). We also compared BHV-1 mapping data to the BHV-5 LR region.

Nucleotide sequence accession number. The BHV-5 genome sequence has been deposited in GenBank under accession no. AY261359.

RESULTS AND DISCUSSION

Genome organization. The BHV-5 genome is 138,390 bp long, 2,518 bp longer than the BHV-1 genome, and contains a 75% G+C base composition. The genome consists of two unique sequences, long or U_L (104,054 bp) and short or U_S (9,548 bp), with the latter being flanked by inverted IR and TR elements of 12,109 bp each. This arrangement corresponds to the D-type herpesviral genome (62).

The BHV-5 origins of DNA replication (ORI) are located in the repeat regions from nucleotide positions 113206 to 113418 and 129595 to 129807. ORI sequences consist of two imperfect AT-rich direct repeats (ORIa and ORIb) which contain herpesvirus consensus sites for the origin-binding protein (84). As in BHV-1, an additional truncated repeat (ORIc) is located 130 bp downstream from ORIb.

Gene characterization. BHV-5 contains 72 genes (Table 1), of which 68 are present as single copies within the unique regions and 2 initiate and are completely located within the repeat regions (BICP4 and BICP22). BHV-5 proteins are most similar to homologues from BHV-1, averaging 82% amino acid identity. All BHV-5 ORFs are present in BHV-1; however, BHV-5 lacks a homologue of UL0.5. Among nonbovine herpesviruses, ORFs of equine herpesviruses (EHV) 1 and 4 are the most similar to those of BHV-5 (28 to 69% amino acid identity). The similarity in gene arrangement and the high percentage of amino acid identity between BHV-5 and previously sequenced alphaherpesviruses support the inclusion of BHV-5 in the α2 subgroup of herpesviruses (62), as was previously suggested by envelope glycoprotein B sequence analysis (80).

U_L region. The U_L region, extending from nucleotide positions 570 to 104623, contains 60 putative genes. Starting from the left end of the genome, the first 58 genes are colinear with their BHV-1 counterparts and represent 73% of the BHV-5 genome. Similarly, the first 53 BHV-5 genes (with the exception of *circ*) are largely colinear with genes UL54 to UL4 of HSV type 1 (HSV-1). Predicted U_L proteins average 84% amino acid identity to BHV-1 homologues, with the most similar (≥95% amino acid identity) involving viral DNA replication and processing (UL5, UL15, UL29, and UL39), tegument (UL14 and UL48), and capsid (UL19). Compared with nonbovine herpesviruses, all BHV-5 capsid proteins and six of eight proteins involved in viral DNA replication or processing are the most conserved (≥60%).

BHV-5 UL49, UL44, UL24, UL11, UL3.5, UL3, UL0.7, LR, and BICP0 are the least conserved U_L genes (≤75%) relative to BHV-1 and are discussed below. Homologues of UL49 and UL3 in HSV-1 and of UL49 in BHV-1 are not essential for virus growth in cultured cells, suggesting a role for these genes in viral pathogenesis and host range (7, 59, 75).

BHV-5 UL44 encodes glycoprotein C (gC), which is not essential for neurovirulence in strain TX89; however, it affects neurotropism and is important for high levels of virus replication and full expression of virulence in the rabbit CNS (21). As alphaherpesvirus gC mediates primary attachment of virus to target cells via binding to surface glycans, variability in the heparin binding sites of BHV-5 gC (gC5) and BHV-1 gC (gC1) likely account for differences in their heparin-binding phenotypes (60). Although gC5 and gC1 are 75% identical, the ami-

TABLE 1. Characterization of BHV-5 genes

ORF no.	ORF name	Position (nt)[a]	Length (aa)[a]	BHV-5 accession no.[b]	BHV-1			Closest non-BHV-1 species				Predicted product and/or function[d]
					Length (aa)	% Identity	Accession no.[b]	Name[c]	Length (aa)	% Identity	Accession no.[b]	
BHV5-01	circ	1167–1901	245		247	84	M96453	EHV-1	257	43	P28988	Myristylated virion protein
BHV5-02	UL54	3482–2274	403		400	82	M96453	EHV-1	470	47	Q05906	Regulates and transports RNA
BHV5-03	UL53	4699–3707	331		332	85	U34593	EHV-1	343	39	AF030027	Glycoprotein K
BHV5-04	UL52	7931–4677	1,085		1,074	83	Z54206	EHV-1	1,081	44	P28962	Component of DNA helicase-primase complex
BHV5-05	UL51	7930–8715	262		243	81	Z54206	PRV	236	49	X87246	Palmitoylated protein (cytoplasm)
BHV5-06	UL50	9821–8859	321		325	83	S62816	EHV-1	326	40	P28892	Deoxyuridine triphosphatase (dUTPase)
BHV5-07	UL49.5	9772–10056	95		96	81	S62816	PRV	98	36	U38547	Glycoprotein N
BHV5-08	UL49	10193–10993	267		258	72	U211137	EHV-1	304	41	X17684	Tegument protein
BHV5-09	UL48	11189–12634	482	AY034598	505	98	Z11610	EHV-4	448	52	AF030027	*trans*-Inducing factor (tegument)
BHV5-10	UL47	12808–15030	741		739	93	P36338	EHV-4	871	35	P28929	Tegument phosphoprotein
BHV5-11	UL46	15166–17367	734		748	82	Z54206	EHV-1	747	40	P28937	Tegument protein
BHV5-12	UL44	19051–17594	486	Z49224	508	75	Z54206	CaHV-1	521	59	Z49225	Glycoprotein C
BHV5-13	UL43	20382–19243	380		378	88	Z54206	EHV-4	403	28	AF030027	Virion protein (membrane)
BHV5-14	UL42	21671–20436	412		408	79	Z54206	PRV	384	38	P36702	Processivity factor for DNA polymerase
BHV5-15	UL41	21714–23288	525		459	91	Z54206	EHV-1	497	48	P28957	Virion host shutoff factor (tegument)
BHV5-16	UL40	24363–23419	315		314	91	Q01319	PRV	303	76	X72087	Ribonucleotide reductase small subunit
BHV5-17	UL39	26785–24386	800		787	97	Z54206	PRV	835	67	X72087	Ribonucleotide reductase large subunit
BHV5-18	UL38	28787–27153	545		474	87	Z54206	EHV-4	462	47	AF030027	Capsid protein
BHV5-19	UL37	28869–32030	1,078		1,024	88	Z54206	EHV-4	1,021	41	AF030027	Tegument protein
BHV5-20	UL36	32133–41744	3,204		3,247	80	Z78205	EHV-1	3,421	40	P28955	Very large tegument protein
BHV5-21	UL35	42351–41977	125		124	87	Z78205	PRV	103	54	AJ276165	Capsid protein
BHV5-22	UL34	43226–42399	276		260	83	Z78205	PRV	260	58	AF301599	Virion protein (membrane)
BHV5-23	UL33	43639–43310	110		108	89	Z78205	EHV-1	162	59	P28953	Capsid packaging protein
BHV5-24	UL32	43620–45413	598		601	89	Z78205	EHV-1	620	53	P28952	Cleavage and packaging protein
BHV5-25	UL31	45409–46545	379		361	84	Z78205	EHV-4	326	64	AF030027	UL34-associated nuclear protein
BHV5-26	UL30	50224–46475	1,250		1,246	90	X94677	EHV-1	1,220	61	P28858	DNA polymerase, catalytic subunit
BHV5-27	UL29	50495–54118	1,208		1,203	95	X94677	EHV-1	1,209	62	P28932	Single-stranded DNA binding protein
BHV5-28	UL28	54358–56805	816		826	90	X94677	EHV-1	775	55	M86664	Cleavage and packaging protein
BHV5-29	UL27	56661–59501	947	AF359759	932	93	P12640	FHV-1	948	58	S49775	Glycoprotein B
BHV5-30	UL26.5	60960–60016	315		308	80	431809	HSV-2	329	38	L37443	Capsid scaffolding protein
BHV5-31	UL26	61872–60016	619		621	83	U31809	EHV-1	646	46	P28936	Capsid maturation serine protease
BHV5-32	UL25	63788–61980	603		598	94	AJ004801	EHV-1	587	58	P28928	DNA packaging virion protein
BHV5-33	UL24	64609–63773	279		293	74	L39072	EHV-1	272	45	P28927	Putative membrane-associated protein
BHV5-34	UL23	64608–65675	356	S56149	359	83	P36226	EHV-4	352	46	AF030027	Thymidine kinase
BHV5-35	UL22	65795–68338	848	AF113752	842	86	P27599	EHV-4	855	33	A21045	Glycoprotein H
BHV5-36	UL21	70346–68538	603		574	77	Z48053	EHV-1	530	38	P28972	Tegument protein
BHV5-37	UL20	70386–71144	253		231	93	Z48053	EHV-1	239	35	P28971	Virion protein (membrane)
BHV5-38	UL19	71227–75399	1,391		1,385	97	Z48053	EHV-1	1,376	69	P28920	Major capsid protein
BHV5-39	UL18	75509–76456	316		316	92	Z48053	EHV-1	314	60	P28921	Capsid protein
BHV5-40	UL17	77927–80047	707		701	90	Z48053	EHV-1	706	44	P28950	Tegument protein
BHV5-41	UL16	80077–81105	343		339	93	Z48053	EHV-1	370	47	P28970	Virion protein
BHV5-42	UL15	82277–76670	737		735	95	Z48053	EHV-1	734	63	P28969	DNA cleavage-packaging protein (terminase)
BHV5-43	UL14	82340–83011	224		222	97	Z48053	EHV-4	321	48	AF030027	Minor tegument protein
BHV5-44	UL13	82923–84407	495		492	90	Z48053	EHV-1	594	38	P28966	Virion serine/threonine protein kinase
BHV5-45	UL12	84407–85867	487		487	88	Z48053	PRV	483	51	X97257	Alkaline exonuclease
BHV5-46	UL11	85822–86121	100		89	73	Z48053	EHV-4	75	48	AF030027	Myristylated protein (tegument)
BHV5-47	UL10	87526–86270	419		438	85	AJ004801	EHV-1	450	38	P28948	Glycoprotein M
BHV5-48	UL9	87647–90115	823		859	89	Z48053	EHV-4	887	57	AF030027	Ori-binding protein
BHV5-49	UL8	90216–92486	757		748	89	AJ004801	EHV-4	751	40	AF030027	Component of DNA helicase-primase complex
BHV5-50	UL7	93451–92549	301		299	88	X91751	EHV-1	303	40	P28945	Virion-associated protein
BHV5-51	UL6	95795–93336	820		688	84	Z48053	PRV	643	62	X97257	Virion protein
BHV5-52	UL5	95551–98064	838		838	99	Z48053	EHV-1	861	64	P28934	Component of DNA helicase-primase complex
BHV5-53	UL4	98088–98651	188		185	86	Z48053	EHV-4	227	39	AF030027	Nuclear protein
BHV5-54	UL3.5	99127–98684	148		126	69	U32173	EHV-4	133	29	AF030027	Virion protein
BHV5-55	UL3	99786–99136	217		204	75	U32173	EHV-1	212	53	P28942	Phosphoprotein
BHV5-56	UL2	100729–99836	298		301	76	AJ004801	PRV	339	58	L13855	Uracil-DNA glycosylase
BHV5-57	UL1	101201–100716	162		158	81	AJ004801	FHV-1	147	39	AF022391	Glycoprotein L
BHV5-58	UL0.7	101005–101607	201		97	41	AJ004801	HHV-6b	1,520	31	AF157706	Unknown
	UL0.5	Not present			87		AJ004801					Unknown
BHV5-59a	LRORF2	102159–102311	51[e]		181	82	M61143					LR region
BHV5-59b	LRORF1	102553–103632	360		336	66	M61143					LR region
BHV5-60	BICP0	104408–102249	720		676	70	P29836	PRV	410	32	A40505	IE transactivator protein with Zn finger
BHV5-61	BICP4	109909–105686	1,406		1,343	75	L14320	EHV-1	1,487	42	P17473	Positive and negative gene regulator
BHV5-62	BICP22	114776–115717	314		300	68	X76943	EHV-1	278	40	Z67986	Transcription factor
BHV5-63	US1.67	117249–116509	247		243	79	AJ004801	EHV-1	272	35	P28984	Virion protein (EHV-1)
BHV5-64	US2	117987–117310	226		220	69	AJ004801	EHV-1	303	37	P32517	Tegument protein
BHV5-65	US3	118110–119441	444		468	79	AJ004801	EHV-4	384	42	AF030027	Virion serine/threonine protein kinase
BHV5-66	US4	119552–120871	440	X99755	444	72	AJ004801	FHV-1	435	32	S72415	Glycoprotein G
BHV5-67	US6	121129–122379	417	U14656	417	98	A25177	CeHV-1	179	84	AF078735	Glycoprotein D
BHV5-68	US7	122532–123692	387		382	78	AJ004801	EHV-1	424	30	P18553	Glycoprotein I
BHV5-69	US8	123984–125780	597	AF208294	575	74	AJ004801	EHV-1	552	41	P24380	Glycoprotein E
BHV5-70	US9	125875–126276	134	AY064172	144	79	Z23068	PRV	106	48	U27487	Virion protein (tegument)
BHV5-71	BICP22	128237–127296	314		300	67	X76943	EHV-1	272	40	Z67986	Transcription factor
BHV5-72	BICP4	133104–137327	1,406		1,343	75	L14320	EHV-1	1,487	42	P17473	Positive and negative gene regulator

[a] aa, amino acids; nt, nucleotides.
[b] Accession numbers are from GenBank or SwissProtein.
[c] Names for viruses: EHV-1, equine herpesvirus 1; PRV, pseudorabies virus; EHV-4, equine herpesvirus 4; CaHV-1, caprine herpesvirus 1; HSV-2, herpes simplex virus type 2; FHV-1, feline herpesvirus 1; HHV-6b, human herpesvirus 6B; CeHV-1, cervid herpesvirus 1.
[d] Function was deduced from the degree of amino acid similarity to products of known genes.
[e] The remaining homologous region is present but contains an in-frame stop and two in-frame frameshifts.

no-terminal third of the proteins (amino acids 1 to 102 and 1 to 123, respectively) differ significantly. Notably, there is a 35-amino-acid deletion in gC5 which removes two potential N-linked glycosylation sites present in gC1 (amino acids 93 and 111) (36) and a gC1-specific epitope (amino acids 103 to 122) (18). Comparison of gC sequences between strain SV507/99 (this report) and strain TX89 (18) reveals substantial differences at the amino-terminal third. However, a more detailed analysis shows that the proteins are 97% identical and that the discrepancy likely results from a missing base at position 480 in the published TX89 sequence.

The homologue of UL24 in HSV-1 is required for efficient replication in TG of mice (50). The homologue of UL11 in HSV-1 encodes a tegument protein with roles in virion envelopment and egress (7). BHV-5 and BHV-1 UL3.5 and UL0.7 are not found in other herpesviruses, and their functions remain unknown. BHV-5 lacks a homologue of BHV-1 UL0.5, which is predicted to encode an 87-amino-acid protein of unknown function.

The latency-related (LR) gene is postulated to contribute via alternative splicing to the LR protein(s) (27, 44), whereas BICP0 encodes a homologue of BHV-1 BICP0, an immediate-early (IE) and early (E) transactivator (95). Given the potential roles of LR and BICP0 genes in virulence and host range, these genes are treated in more detail below (see "IE genes" and "The LR region," below).

U_S region. The U_S region, extending from positions 116733 to 126280, contains eight genes (US1.67, 2 to 4, and 6 to 9), four of which have been previously sequenced (1, 20, 22, 31). BHV-5 U_S genes exhibit 69 to 98% amino acid identity (average, 79%) and overall less conservation to BHV-1 homologues than those within the U_L region (84% average amino acid identity) (58). BHV-5 US2 to US9 are syntenic with homologues in HSV-1, with the exception of the US5 homologue, which is lacking in BHV-5.

The two BHV-5 genes located at the ends of the U_S are likely significant for virus-host interactions. The gene at the U_S-IR boundary, US1.67, contains the 75 carboxy-terminal amino acids within the IR and is homologous to ORFs found in other members of the α2 herpesvirus subgroup. Notably, the homologue of US1.67 in EHV-1 is a virulence determinant and is involved in egress of viral nucleocapsids (70, 71, 89). The gene at the U_S-TR boundary, US9, is essential for neurovirulence in the TX89 strain. Following intranasal inoculation of rabbits, a TX89 strain US9 deletion mutant failed to invade the CNS mainly due to an inability of the virus to spread to the olfactory bulb via anterograde transport (22).

BHV-5 US2, US4, and US8 are the least-conserved U_S ORFs ($\leq$75%) in comparison with BHV-1. The US2 homologue in HSV-1 and HSV-2 seems dispensable in tissue culture and is not involved in HSV-2 neuropathogenesis in mice (52, 61). BHV-5 US4 encodes a glycosaminoglycan-associated protein (31) and is 72% identical to BHV-1 gG, which is involved in cell-cell virus transmission in vitro (69) and in prevention of apoptosis in certain cell lines (68). BHV-5 US8 encodes gE, which is important for neurovirulence in rabbits. Deletion of BHV-5 US8 or its substitution by BHV-1 gE resulted in viruses that replicated and spread much less efficiently in the rabbit brain than revertant or wild-type viruses (20).

Repeats. The IR and TR regions, located at nucleotide positions 104624 to 116732 and 126281 to 138389, respectively, are 12,109 bp in length. Each repeat contains two genes, BICP4 and BICP22, which are 75 and 68% identical to BHV-1 BICP4 and BICP22, respectively (see "IE genes," below). Among nonbovine herpesviruses, genes of EHV-1 are the most similar to BICP4 (42% amino acid identity) and BICP22 (40% amino acid identity). Interestingly, a homologue of the γ1 34.5 gene, a neurovirulence determinant of HSV-1, is not present in BHV-5 (79).

A 4,867-bp noncoding region separates BICP4 from BICP22 start codons. Overall, this region is 56% identical to the homologous BHV-1 intergenic region and contains transcriptional regulatory elements for the flanking IE genes and the origins of DNA replication.

IE genes. Herpesvirus IE genes are critical regulators of viral gene expression. BHV-5 BICP0, BICP4, and BICP22, the homologues of BHV-1 IE genes BICP0, BICP4, and BICP22, are relatively less conserved than other classes of viral genes (Table 1).

In BHV-1, the BICP0 gene is transcribed at IE and E times postinfection from two separate promoters, leading to accumulation of 2.9- and 2.6-kb RNAs, respectively (94, 95). The IE promoter also controls BICP4 expression at IE times postinfection. A BICP0-specific transcript of similar size has been detected in BHV-5-infected cells at IE times (96). Several sequence elements involved in IE transcription and RNA processing of BHV-1 BICP0, including the TATA box (BHV-5 position 110799), major transcription start site (position 110766), donor and acceptor splice sites (positions 110417 and 104413, respectively), and polyadenylation signal (position 102150), are conserved in BHV-5. Further, potential TATA and SP1 sites are located at -39 and -71 relative to the first BICP0 codon, respectively, suggesting a similar promoter may direct E expression of BHV-5 BICP0 as in BHV-1 (95). Taken together, these features suggest that common mechanisms control BICP0 transcription in the two viruses.

BHV-5 BICP0 shares 70% amino acid identity with BHV-1 BICP0. Most amino acid differences are found at the carboxy half of the protein, a region which plays a role in subcellular localization of ICP0-like proteins (33, 47). BHV-5 BICP0 is 44 amino acids longer that its BHV-1 counterpart, including several small amino acid insertions and a 30-amino-acid extension at the carboxy terminus. This difference is likely responsible for the slightly larger size of IE transcripts originated from BHV-5 BICP0 relative to those from BHV-1 (96). Both BHV-5 and BHV-1 BICP0s are most similar in the amino half, which contains a conserved acidic cluster (amino acids 273 to 323 in BHV-5) and a C_3HC_4 zinc ring finger (amino acids 22 to 59). However, the region between amino acids 119 and 145 (110 to 155 in BHV-1) is poorly conserved and includes a 20-amino-acid deletion in BHV-5. In BHV-1, the BICP0 ring finger has been implicated in promoting transactivation, stimulation of productive infection, and cytotoxicity (47). It is not known if BHV-1 BICP0 contributes to virus pathogenesis; however, a point mutation in HSV-1 ICP0 significantly reduced neuroinvasiveness of an HSV-1 neurovirulent strain after peripheral inoculation of mice (90).

BHV-5 BICP4 encodes the homologue of BHV-1 BICP4, an IE transactivator and transrepressor (84). Based on sequence

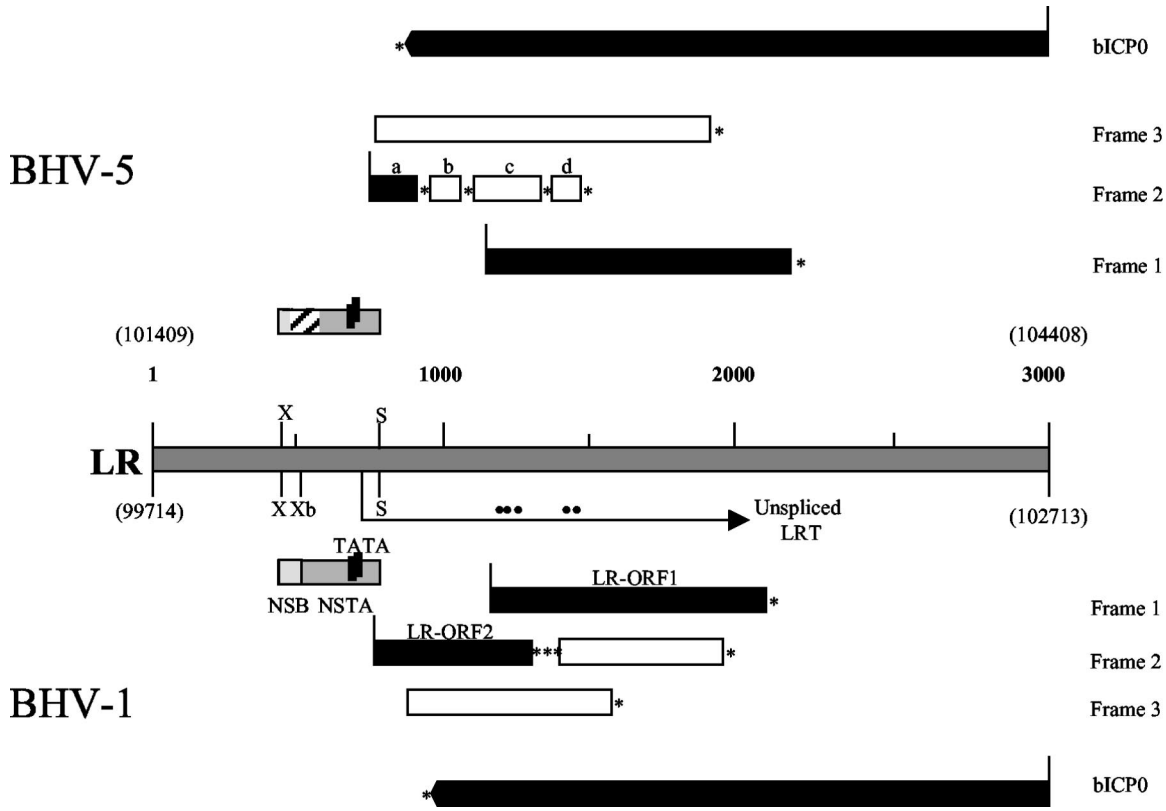

FIG. 1. Comparison of the LR regions of BHV-5 and BHV-1. The LR nucleotide positions are given above (BHV-5) and below (BHV-1) the central stippled box, and the relative nucleotide positions are in bold (1 to 3,000 bp). Restriction sites are *Xho*I (X), *Xba*I (Xb), and *Sph*I (S). The dots above the BHV-1 LRT represent the positions of splice donor signals (27). Methionine- and non-methionine-initiated ORFs are represented by black and open boxes, respectively. Asterisks indicate the positions of in-frame stop codons. BHV-1 neuron-specific transcriptional regulatory regions NSB and NSTA are represented by boxes between *Xho*I and *Sph*I sites. The hatched box in the BHV-5 regulatory region represents deleted sequences. The structure of BHV-5 frames 1 and 2 is discussed in the text and shown in detail below in Fig. 2A and B.

homologies, alphaherpesvirus ICP4 orthologs are divided into regions I to V from the amino to the carboxy terminus (13, 17, 43, 84). BHV-5 and BHV-1 ICP4 are most similar (90 to 100% amino acid identity) within regions II and IV. When compared with HSV-1, BHV-5 BICP4 regions II and IV are also the most similar (>50% amino acid identity). HSV-1 ICP4 region II is involved in homodimerization and DNA binding, whereas region IV is required for efficient transactivation (see reference 13 and references therein). BHV-5 BICP4 is 63 amino acids longer than BHV-1 BICP4, and this may be responsible for the slightly larger size of IE transcripts originated from BHV-5 BICP4 relative to their BHV-1 counterparts (96). The difference in length is partially due to a 47-amino-acid insertion in BHV-5 BICP4 region III, which includes a unique stretch of the dipeptide DG between two glutamic acid-rich clusters unique to bovine alphaherpesvirus ICP4s. BHV-5 BICP22 encodes the homologue of the BHV-1 IE and late transrepressor BICP22 (85). Most differences are observed in the carboxy halves of the proteins (48% amino acid identity versus 82% in the amino termini). This region contains 30 serines in BHV-5 and 16 in BHV-1 in a tract of 35 residues.

The LR region. The BHV-1 LR region is the only transcriptionally active region in latently infected neurons and is hypothesized to play a role in latent infections (44, 76). In vitro, the BHV-1 LR gene inhibits cell cycle progression (83), BICP0-dependent activation of productive infection (39), and apoptosis (23). Within the LR gene there exist two major ORFs designated ORF1 and ORF2, where ORF1 is completely overlapped by BICP0 (56). Using an antibody against the amino terminus of ORF2, an LR protein was detected in lytically infected cells and in latently infected TG neurons (44, 51). Infection of cattle with mutant BHV-1 containing three stop codons that should prevent LR protein expression leads to a reduced ability of virus to replicate in the eye during acute infection (48), lower levels of viral DNA in latently infected TG, and a severe defect in virus reactivation after dexamethasone treatment of latently infected animals (49).

A complex pattern of alternative splicing in the LR transcript (LRT) has been described (27). LRT splicing varied according to the source of LR RNA (cell lines or TG), the time postinfection, and the type of RNA [poly(A)$^+$ or poly(A)$^-$] used for the experiments. The splicing pattern allows for different combinations between methionine-initiated ORFs and between methionine-initiated and non-methionine-initiated ORFs which may result in different protein isoforms.

Figure 1 summarizes major differences between BHV-5 and BHV-1 in the LR region. Whereas both viruses contain ORF1 in frame 1 (66% amino acid identity), the structure of BHV-5 frame 2 departs from that of BHV-1. In BHV-1, frame 2 contains an ORF (LRORF2) which could encode a 181-amino-

A

```
                10         20         30         40         50         60         70         80
BHV-1  MRDLGHKSPAHARAVTFGLGMTAATAARKLSMQAGGRKYTAVRCGGLRVAEWVGGEP-AAAIIAARRCRRCSGRAARPGSGPG
       ||   ||||||||||||||||||:|||:||||||||||::|  ||||||    |:  |||| ||| || ||||| |||| |
BHV-5  MRCRRHKSPAHARAVTFGLGMTAAVAARELSMQAGGRKYAVVWRGGLRVAG*GRGRALAAAITAARSRRRYSGRAAWPGSGLG
         102180     102210     102240     102270     102300     102330     102360     102390

                90        100        110        120        130        140        150        160
BHV-1  DRLAAAPGPRCCGG-GSPGGFLAPRRPLPFRCAASSAPSSRGRGPGRPAPWRSLA--FSPGRGCHCGRPRPGRLRPGPERRPA
        | ||| |||| || || || |||||| |||||| |||||||||||||||||  |  ::|:|     |||    |  |  ||:
BHV-5  ARWAAALGPRCRGG/GSLGGVLAPRRPPPFRCAAFSAPSSRGRGPGRPAPWRPRASCLGPSRLRGWLRPRWPRPGPPPSPRPT
       102420     102450     102480     102510     102540     102570     102600     102630

           170
BHV-1  GGRRQGRGRRETGSGLGWK*
       |:||:| ||: :|:|||
BHV-5  GARRRGCGRQGAGAGLGLG~~~
       102660     102690
```

B

```
                10         20         30         40         50         60         70         80
BHV-1 MALAGLLSRPGLPLRPTSARAAPARAGA--PARRGSAAGARPAGDGVGARVEIAAAVLVAAAVTAGLGAELRATEGAAAAAVT
      || |||||||  | | :||::| | |::  ||:||| ||:||||:| || :  :| : :|||  || || :|| | :||||::
BHV-5 MAPAGLLSRPEPPSRLASAQVASAGAATEPPAHRGSPAGVRPAGSGSGAGAGAGAWAETAAA--AGPGAGVRAMEDSAAAAAA
         102580     102610     102640     102670     102700     102730     102760     102790

                90        100                  110        120        130        140        150
BHV-1 AAAAAGVPICAPAEAGPGAAAGAGS----------AGRAGAFAVRSITVEMGAGAGAGAGAGVGARSNSVLLLRRACTRGGPA
      ||::|:|     | :|  |:|:||:          |||||| ||||| |||||||||:||     |||:|| ||||||||||||
BHV-5 AATVAAVVAADAAASGARAGAAAGTPGRARAAPRPAGRAGASAVRSI-VEMGAGAGVGA----RARSTSV-LLRRACTRGGPA
         102820     102850     102880     102910     102940         102970     103000

         160            170            180         190        200        210        220
BHV-1 SPSSLARRSPRPSSLP----APQPAVSP------AATP-KPAP-LPSSLGSGLHARACAAGAARGAAGNRGSRLPPRLRELGG
      |||| |||| || | |    | :||:||      |||| |||| ||||| || |||||||||:  |||: |:|  ||||  ||
BHV-5 SPSSPARRSSRPPSPPVTTTASHPAASPPAGSPPAATPGKPAPPLPSSLRSGPHARACAAGAGCDAAGGWGARRSPRLRGRGG
   103030     103060     103090     103120     103150     103180     103210     103240     103270

         230        240        250        260        270        280        290        300
BHV-1 AETAAARPQ-GRRASDRGAGCTAGVCRPLVLTYCLSAGRRARAAPDAPRVRLDSTSASVSGSVSVSSMAVRSEVLSPDDDSSD
      ||||||||  ||||||| || |||||||||||||| |||||:|| |||||   |||| | | ||||||||||||||||||||
BHV-5 AETAAARPPLGRRASDRDAGRTAGVCRPLVLTYCLSPGRRARVAPAAPRVRRAPTSASDSDSDSVSSMAVRSEVLSPDDDSSD
         103300     103330     103360     103390     103420     103450     103480     103510

        310            320         330
BHV-1 SDQESSS----EPSESESE--VSRNTTPRPPGGADC*
      |||||||    ||||||||  |||||||||||||
BHV-5 SDQESSSSSSSEPSESESESEVSRNTTPRPPGGADR*
      103540     103570     103600
```

FIG. 2. (A) Alignment of BHV-1 LRORF2 with translated BHV-5 genomic sequence using tfastx, indicating the homologous BHV-5 ORF in frame 2 (boxed), stop codons (*), frameshift to BHV-5 frame 3 (/), conserved substitutions (:), identities (|), and remaining open sequence in BHV-5 frame 3 (~~~). Total aligned sequence (182 amino acids) is 69% identical with BHV-1 LRORF2. (B) Alignment of BHV-1 LRORF1 with translated BHV-5 sequence using tfastx. Identities and substitutions are labeled as for panel A. Total aligned sequence (367 amino acids) is 66% identical with BHV-1 LRORF1. (C) Alignment of BHV-1 and BHV-5 LR regulatory sequences represented by the hatched box in Fig. 1, using FASTA. In BHV-1, the underlined sequence 1 was specifically protected from exonuclease digestion by ganglionic nuclear factors (25); underlined sequence 2 was protected from exonuclease digestion by neuroblastoma nuclear factors (11). Potential TATA and CAT boxes are shown in bold.

acid protein and a downstream reading frame without an initiating methionine. In BHV-5, frame 2 is interrupted four times, resulting in reading frames *a* to *d* (Fig. 1), with reading frame *a* being the only one to be methionine initiated. Reading frame *a*, which is truncated at 51 amino acids, and reading frame *b* are 82 and 75% identical to the corresponding regions in BHV-1 ORF2, respectively, while reading frames *c* and *d* are frameshifted. Interestingly, all splicing donor sites mapped in

C

```
        430       440       450  XhoI 460       470       480       490       500       510
BHV1    ACCCAGACGCTCGGCACTTAGCTCTCGAGTGATTGCCTGGCTGCTTGCCCCGTTTGCGTGGAATGAGGCCAAACAAGCATTT
        || ||||||||| ||||||||||||||||||| || ||||| || |||||| |                1
BHV5    ACTCAGACGCTCTGCACTTAGCTCTCGAGTGAATGTCTGGCCGCCTGCCCCAT----------------------------
           480       490       500       510       520

                520   XbaI530       540       550       560       570       580       590
BHV1    GGCCCCTGTTTGTCTAGACGGGGCTTTGTGTTTTTCCCACCCCTGCCCTTGCCCCTGGCCGCCTGGCCGGCCGGCTAAAGTA
          |||                 ||| ||||   2          | ||  | ||  ||| | ||||  |||| |    || |
BHV5    --CCC-----------------GCTATGTG--------------GGCCAGGGCC--GGCAGACTGG--GGCCCGTGCCAG-A
          530                                     540         550         560       570

            600       610       620       630       640       650       660       670
BHV1    TAGGCCAGACCAAACCCCCCGCAGCAG-CTCTGCTCGCTGGCGAGACCCCAAAGCGCCAATTGTGGCATA-TTTCCGGGTCT
        |  ||    ||   ||| |||| |||| ||||||| |||||||    ||||||| ||    |||||| || ||||| ||||
BHV5    TCCGCT---CCC--CCCTCCGCGGCAGTCTCTGCTAGCTGGCGGAGTCCCAAAGTGCTGGCTGTGGCCTAGTTTCCCGGTCC
                580         590       600       610       620       630       640      650

            680       690       700       710       720
BHV1    -GACCGCGCCTGCACTTGCCTTTGGCATTAATATTTCAGAGTTTAATATTACAG
         | ||||||| |||||||  |||  ||||||||||||||||||||||||||||
BHV5    CGGCCGCGCCCGCACTTGT-TTTCACATTAATATTTCAGAGTTTAATATTACAG
            660       670       680       690       700
```

FIG. 2—*Continued.*

the BHV-1 LR transcript fall downstream of the carboxy terminus of frame *b*, suggesting that if BHV-5 proteins were made from this region they would differ substantially from BHV-1 LR products. A detailed comparison of LRORF2 and LRORF1 amino acid sequences between the two viruses is shown in Fig. 2A and B.

The promoter which drives BHV-1 LR transcription is contained near the 5′ terminus of the LR gene (53) and includes two neuron-specific regulatory regions designated the neuron-specific binding protein region and neuron-specific transcription activator (NSB and NSTA, respectively) (Fig. 1) (11, 25). While regions in the vicinity of the TATA box are conserved between BHV-5 and BHV-1, nucleotide sequences 5′ of NSTA are poorly conserved or lacking in BHV-5 (Fig. 2C). These sequences were shown to be important for efficient enhancer activity of the BHV-1 LR promoter in neuroblastoma cells and for binding of nuclear factors (11). The NSB region is 72 bp in length, specifically binds bovine ganglionic nuclear factors, and is required for maximal BHV-1 LR promoter activity in neuroblastoma cells (25). Almost 70% of the NSB region is deleted in BHV-5, including a 20-bp region responsible for binding to neuron-specific factors (25) (Fig. 2C) and a latency-specific transcription start site (44).

In summary, the BHV-5 LR region differs substantially from the BHV-1 LR region in both coding and transcriptionally regulatory regions. Given the potential significance of this region in viral latency and/or host range and the differential pathogenesis of these closely related viruses, it is likely that these differences are of biological significance for aspects of virus-neuron-host interactions.

Conclusions. Although BHV-5 and BHV-1 are closely related viruses, only BHV-5 causes CNS disease. The molecular basis for the differential pathogenesis remains unknown but probably arises from multiple genetic contributions. The complete BHV-5 genome provides a framework from which comparisons between BHV-5 and BHV-1 pathogenesis may be made. Strategies based on the construction of chimeric viruses will certainly contribute to our overall understanding of pathogen-host interactions and the evolution of herpesvirus virulence.

REFERENCES

1. **Abdelmagid, O. Y., H. C. Minocha, J. K. Collins, and S. I. Chowdhury.** 1995. Fine mapping of bovine herpesvirus-1 (BHV-1) glycoprotein D (gD) neutralizing epitopes by type-specific monoclonal antibodies and sequence comparison with BHV-5 gD. Virology **206:**242–253.
2. **Afonso, C. L., E. R. Tulman, Z. Lu, L. Zsak, G. F. Kutish, and D. L. Rock.** 2000. The genome of fowlpox virus. J. Virol. **74:**3815–3831.
3. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
4. **Altschul, S. F., T. L. Madden, A. A. Schaffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman.** 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. **25:**3389–3402.
5. **Ashbaugh, S. E., K. E. Thompson, E. B. Belknap, P. C. Schultheiss, S. Chowdhury, and J. K. Collins.** 1997. Specific detection of shedding and latency of bovine herpesvirus 1 and 5 using a nested polymerase chain reaction. J. Vet. Diagn. Investig. **9:**387–394.
6. **Bagust, T. J., and L. Clark.** 1972. Pathogenesis of meningo-encephalitis produced in calves by infectious bovine rhinotracheitis herpesvirus. J. Comp. Pathol. **82:**375–383.
7. **Baines, J. D., and B. Roizman.** 1991. The open reading frames UL3, UL4, UL10, and UL16 are dispensable for the replication of herpes simplex virus 1 in cell culture. J. Virol. **65:**938–944.
8. **Barenfus, M., C. A. Delli Quadri, R. W. McIntyre, and R. J. Schroeder.** 1963. Isolation of infectious bovine rhinotracheitis virus from calves with meningoencephalitis. J. Am. Vet. Med. Assoc. **143:**725–728.
9. **Bartha, A., G. Haidu, P. Aldassy, and G. Paczolay.** 1969. Occurrence of encephalomyelitis caused by infectious bovine rhinotracheitis virus in calves in Hungary. Acta Vet. Acad. Sci. Hungaricae **19:**145–151.
10. **Belknap, E. B., J. K. Collins, V. K. Ayers, and P. C. Schultheiss.** 1994. Experimental infection of neonatal calves with neurovirulent bovine herpesvirus type 1.3. Vet. Pathol. **31:**358–365.
11. **Bratanich, A. C., and C. J. Jones.** 1992. Localization of *cis*-acting sequences in the latency-related promoter of bovine herpesvirus 1 which are regulated by neuronal cell type factors and immediate-early genes. J. Virol. **66:**6099–6106.
12. **Brendel, V., P. Bucher, I. R. Nourbakhsh, B. E. Blaisdell, and S. Karlin.**

1992. Methods and algorithms for statistical analysis of protein sequences. Proc. Natl. Acad. Sci. USA **89:**2002–2006.
13. **Bruce, J. W., and K. W. Wilcox.** 2002. Identification of a motif in the C terminus of herpes simplex virus regulatory protein ICP4 that contributes to activation of transcription. J. Virol. **76:**195–207.
14. **Burks, C.** 1999. Molecular Biology Database list. Nucleic Acids Res. **27:**1–9.
15. **Carrillo, B. J., A. Ambrosi, A. A. Schudel, M. Vazquez, E. Dahme, and A. Pospischil.** 1983. Meningoencephalitis caused by IBR virus in calves in Argentina. Zentbl. Vet. Med. B **30:**327–332.
16. **Cascio, K. E., E. B. Belknap, P. C. Schultheiss, A. D. Ames, and J. K. Collins.** 1999. Encephalitis induced by bovine herpesvirus 5 and protection by prior vaccination or infection with bovine herpesvirus 1. J. Vet. Diagn. Investig. **11:**134–139.
17. **Cheung, A. K.** 1989. DNA nucleotide sequence analysis of the immediate-early gene of pseudorabies virus. Nucleic Acids Res. **17:**4637–4646.
18. **Chowdhury, S. I.** 1995. Molecular basis of antigenic variation between the glycoproteins C of respiratory bovine herpesvirus 1 (BHV-1) and neurovirulent BHV-5. Virology **213:**558–568.
19. **Chowdhury, S. I., B. J. Lee, D. Mosier, J.-H. Sur, F. A. Osorio, G. Kennedy, and M. L. Weiss.** 1997. Neuropathology of bovine herpesvirus type 5 (BHV-5) meningo-encephalitis in a rabbit seizure model. J. Comp. Pathol. **117:**295–310.
20. **Chowdhury, S. I., B. J. Lee, A. Ozkul, and M. L. Weiss.** 2000. Bovine herpesvirus 5 glycoprotein E is important for neuroinvasiveness and neurovirulence in the olfactory pathway of the rabbit. J. Virol. **74:**2094–2106.
21. **Chowdhury, S. I., B. J. Lee, M. Onderci, M. L. Weiss, and D. Mosier.** 2000. Neurovirulence of glycoprotein C (gC)-deleted bovine herpesvirus type-5 (BHV-5) and BHV-5 expressing BHV-1 gC in a rabbit seizure model. J. Neurovirol. **6:**284–295.
22. **Chowdhury, S. I., M. Onderci, P. S. Bhattacharjee, A. Al-Mubarak, M. L. Weiss, and Y. Zhou.** 2002. Bovine herpesvirus 5 (BHV-5) U_s9 is essential for BHV-5 neuropathogenesis. J. Virol. **76:**3839–3851.
23. **Ciacci-Zanella, J., M. Stone, G. Henderson, and C. Jones.** 1999. The latency-related gene of bovine herpesvirus 1 inhibits programmed cell death. J. Virol. **73:**9734–9740.
24. **Collins, J. K., V. K. Ayers, C. A. Whetstone, and S. van Drunen Little-van den Hurk.** 1993. Antigenic differences between the major glycoproteins of bovine herpesvirus type 1.1 and bovine encephalitis herpesvirus type 1.3. J. Gen. Virol. **74:**509–1517.
25. **Delhon, G., and C. Jones.** 1997. Identification of DNA sequences in the latency related promoter of bovine herpes virus type 1 which are bound by neuronal specific factors. Virus Res. **51:**93–103.
26. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12:**387–395.
27. **Devireddy, L. R., and C. Jones.** 1998. Alternative splicing of the latency-related transcript of bovine herpesvirus 1 yields RNAs containing unique open reading frames. J. Virol. **72:**7294–7301.
28. **d'Offay, J. M., R. E. Mock, and R. W. Fulton.** 1993. Isolation and characterization of encephalitic bovine herpesvirus type 1 isolates from cattle in North America. Am. J. Vet. Res. **54:**534–539.
29. **Dolan, A., F. E. Jamieson, C. Cunningham, B. C. Barnett, and D. J. McGeoch.** 1998. The genome sequence of herpes simplex virus type 2. J. Virol. **72:**2010–2021.
30. **Down, T. A., and T. J. P. Hubbard.** 2002. Computational detection and location of transcription start sites in mammalian genomic DNA. Genome Res. **12:**458–461.
31. **Engelhardt, T., and G. M. Keil.** 1996. Identification and characterization of the bovine herpesvirus 5 US4 gene and gene products. Virology **225:**126–135.
32. **Engels, M., C. Giuliani, P. Wild, T. M. Beck, E. Loepfe, and R. Wyler.** 1986. The genome of bovine herpesvirus 1 (BHV-1) strains exhibiting a neuropathogenic potential compared to known BHV-1 strains by restriction site mapping and cross-hybridization. Virus Res. **6:**57–73.
33. **Everett, R. D.** 1988. Analysis of the functional domains of herpes simplex virus type 1 immediate early polypeptide Vmw110. J. Mol. Biol. **202:**87–96.
34. **Ewing, B., and P. Green.** 1998. Base-calling of automated sequencer traces using *Phred*. II. Error probabilities. Genome Res. **8:**186–194.
35. **Ewing, B., L. Hillier, M. C. Wendl, and P. Green.** 1998. Base calling of automated sequencer traces using *Phred*. I. Accuracy assessment. Genome Res. **8:**175–185.
36. **Fitzpatrick, D. R., L. A. Babiuk, and T. J. Zamb.** 1989. Nucleotide sequence of bovine herpesvirus type 1 glycoprotein gIII, a structural model for gIII as a new member of the immunoglobulin superfamily, and implications for the homologous glycoproteins of other herpesviruses. Virology **173:**46–57.
37. **French, E. L.** 1962. A specific virus encephalitis in calves: isolation and characterization of the causal agent. Aust. Vet. J. **38:**216–221.
38. **French, E. L.** 1962. Relationship between infectious rhinotracheitis (IBR) virus and a virus isolated from calves with encephalitis. Aust. Vet. J. **38:**555–556.
39. **Geiser, V., M. Inman, Y. Zhang, and C. Jones.** 2002. The latency-related gene of bovine herpesvirus-1 can inhibit the ability of bICP0 to activate productive infection. J. Gen. Virol. **83:**2965–2971.
40. **Gillette, K., V. Misra, and A. Bratanich.** 2002. Sequence analysis of the alpha trans-inducing factor of bovine herpesvirus type 5 (BHV-5). Virus Genes **24:**149–152.
41. **Gordon, D., C. Abajian, and P. Green.** 1998. *Consed*: a graphical tool for sequence finishing. Genome Res. **8:**192–202.
42. **Gough, A., and D. James.** 1975. Isolation of IBR virus from heifer with meningo-encephalitis in calves. Can. Vet. J. **16:**313–314.
43. **Grundy, F. J., R. P. Baumann, and D. J. O'Callaghan.** 1989. DNA sequence and comparative analysis of the equine herpesvirus type 1 immediate early gene. Virology **172:**223–236.
44. **Hossain, A., L. M. Schang, and C. Jones.** 1995. Identification of gene products encoded by the latency-related gene of bovine herpesvirus 1. J. Virol. **69:**5345–5352.
45. **Huang, X., and A. Madam.** 1999. CAP3: a DNA sequence assembly program. Genome Res. **9:**868–877.
46. **Husak, P. J., T. Kuo, and L. W. Enquist.** 2000. Pseudorabies virus membrane proteins gI and gE facilitate anterograde spread of infection in projection-specific neurons in the rat. J. Virol. **74:**10975–10983.
47. **Inman, M., Y. Zhang, V. Geiser, and C. Jones.** 2001. The zinc finger in the bICP0 protein encoded by bovine herpesvirus-1 mediates toxicity and activates productive infection. J. Gen. Virol. **82:**483–492.
48. **Inman, M., L. Lovato, A. Doster, and C. Jones.** 2001. A mutation in the latency-related gene of bovine herpesvirus 1 leads to impaired ocular shedding in acutely infected calves. J. Virol. **75:**8507–8515.
49. **Inman, M., L. Lovato, A. Doster, and C. Jones.** 2002. A mutation in the latency-related gene of bovine herpesvirus 1 disrupts the latency-reactivation cycle in calves. J. Virol. **76:**6771–6779.
50. **Jacobson, J. G., S. H. Chen, W. J. Cook, M. F. Kramer, and D. M. Coen.** 1998. Importance of the herpes simplex virus UL24 gene for productive ganglionic infection in mice. Virology **242:**161–169.
51. **Jiang, Y., A. Hossain, M. T. Winkler, T. Holt, A. Doster, and C. Jones.** 1998. A protein encoded by the latency-related gene of bovine herpesvirus 1 is expressed in trigeminal ganglionic neurons of latently infected cattle and interacts with cyclin-dependent kinase 2 during productive infection. J. Virol. **72:**8133–8142.
52. **Jiang, Y. M., H. Yamada, F. Goshima, T. Daikoku, S. Oshima, K. Wada, and Y. Nishiyama.** 1998. Characterization of the herpes simplex virus type 2 (HSV-2) US2 gene product and a US2-deficient HSV-2 mutant. J. Gen. Virol. **79:**2777–2784.
53. **Jones, C., G. Delhon, A. Bratanich, G. Kutish, and D. Rock.** 1990. Analysis of the transcriptional promoter which regulates the latency-related transcript of bovine herpesvirus 1. J. Virol. **64:**1164–1170.
54. **Jones, D. T., W. R. Taylor, and J. M. Thornton.** 1994. A model recognition approach to the prediction of all-helical membrane protein structure and topology. Biochemistry **33:**3038–3049.
55. **Krogh, A.** 1997. Two methods for improving performance of an HMM and their application for gene finding, p. 179–186. *In* T. Gaasterland et al. (ed.), Proceedings of the Fifth International Conference on Intelligent Systems for Molecular Biology. AAAI Press, Menlo Park, Calif.
56. **Kutish, G., T. Mainprize, and D. L. Rock.** 1990. Characterization of the latency-related transcriptionally active region of the bovine herpesvirus 1 genome. J. Virol. **64:**5730–5737.
57. **Lee, B. J., M. L. Weiss, D. Mosier, and S. I. Chowdhury.** 1999. Spread of bovine herpesvirus type 5 (BHV-5) in the rabbit brain after intranasal inoculation. J. Neurovirol. **5:**474–484.
58. **Leung-Tack, P., J.-C. Audonnet, and M. Riviere.** 1994. The complete DNA sequence and the genetic organization of the short unique region (Us) of the bovine herpesvirus type 1 (ST strain). Virology **199:**409–421.
59. **Liang, X., B. Chow, Y. Li, C. Raggo, D. Yoo, S. Attah-Poku, and L. A. Babiuk.** 1995. Characterization of bovine herpesvirus 1 UL49 homolog gene and product: bovine herpesvirus 1 UL49 homolog is dispensable for virus growth. J. Virol. **69:**3863–3867.
60. **Liman, A., M. Engels, G. Meyer, and M. Ackermann.** 2000. Glycoprotein C of bovine herpesvirus 5 (BHV-5) confers a distinct heparin-binding phenotype to BHV-1. Arch. Virol. **145:**2047–2059.
61. **Longnecker, R., and B. Roizman.** 1987. Clustering of genes dispensable for growth in culture in the S component of the HSV-1 genome. Science **236:** 573–576.
62. **McGeoch, D. J., and A. J. Davison.** 1999. The molecular evolutionary history of the herpesviruses, p. 441. *In* E. Domingo, R. Webster, and J. Holland (ed.), Origin and evolution of viruses. Academic Press, London, England.
63. **Metzler, A. E., A. A. Schudel, and M. Engels.** 1986. Bovine herpesvirus 1: molecular and antigenic characteristics of variant viruses isolated from calves with neurological disease. Arch. Virol. **87:**205–217.
64. **Meyer, G., O. Bare, and E. Thiry.** 1999. Identification and characterization of bovine herpesvirus type 5 glycoprotein H and gene products. J. Gen. Virol. **80:**2849–2859.
65. **Meyer, G., M. Lemaire, C. Ros, K. Belak, A. Gabriel, D. Cassart, F. Coignoul, S. Belak, and E. Thiry.** 2001. Comparative pathogenesis of acute and latent infections of calves with bovine herpesvirus types 1 and 5. Arch. Virol. **146:**633–652.
66. **Moretti, B., Z. Orfei, G. Mondino, and A. Perschino.** 1964. Infectious bovine

rhinotracheitis clinical observations and isolation of virus. Vet. Italiana **15:** 676.

67. **Mulder, W. A. M., L. Jacobs, J. Priem, G. L. Kok, F. Wagenaar, T. G. Kimman, and J. M. A. Pol.** 1994. Glycoprotein gE-negative pseudorabies virus has a reduced capability to infect second- and third-order neurons of the olfactory and the trigeminal routes in the porcine central nervous system. J. Gen. Virol. **75:**3095–3106.
68. **Nakamichi, K., D. Kuroki, Y. Matsumoto, and H. Otsuka.** 2001. Bovine herpesvirus 1 glycoprotein G is required for prevention of apoptosis and efficient viral growth in rabbit kidney cells. Virology **279:**488–498.
69. **Nakamichi, K., Y. Matsumoto, and H. Otsuka.** 2002. Bovine herpesvirus 1 glycoprotein G is necessary to maintain cell-to-cell junctional adherence among infected cells. Virology **294:**22–30.
70. **Osterrieder, N., A. Neubauer, C. Brandmüller, O.-R. Kaaden, and D. J. O'Callaghan.** 1996. The equine herpesvirus 1 IR6 protein influences virus growth at elevated temperature and is a major determinant of virulence. Virology **226:**243–251.
71. **Osterrieder, N., A. Neubauer, C. Brandmüller, O.-R. Kaaden, and D. J. O'Callaghan.** 1998. The equine herpesvirus 1 IR6 protein that colocalizes with nuclear lamins is involved in nucleocapsid egress and migrates from cell to cell independently of virus infection. J. Virol. **72:**9806–9817.
72. **Pearson, W. R.** 1990. Rapid and sensitive sequence comparison with FASTP and FASTA. Methods Enzymol. **183:**63–98.
73. **Perez, S. E., G. Bretschneider, M. R. Leunda, E. A. Osorio, E. F. Flores, and A. C. Odeon.** 2002. Primary infection, latency, and reactivation of bovine herpesvirus type 5 in the bovine nervous system. Vet. Pathol. **39:**437–444.
74. **Pertea, M., X. Lin, and S. L. Salzberg.** 2001. A new computational method for splice site prediction. Nucleic Acids Res. **29:**1185–1190.
75. **Pomeranz, L. E., and J. A. Blaho.** 1999. Modified VP22 localizes to the cell nucleus during synchronized herpes simplex virus type 1 infection. J. Virol. **73:**6769–6781.
76. **Rock, D. L., S. L. Beam, and J. E. Mayfield.** 1987. Mapping bovine herpesvirus type 1 latency-related RNA in trigeminal ganglia of latently infected rabbits by in situ hybridization. J. Virol. **61:**3827–3831.
77. **Roehe, P. M., T. C. Silva, N. B. Nardi, D. G. Oliveira, and J. C. A. Rosa.** 1997. Diferenciaco entre o vírus da rinotraqueite infecciosa bovina (BHV-1) e o herpesvirus da encefalite bovina (BHV-5). Pesq. Vet. Bras. **17:**41–44.
78. **Roizman, B., R. C. Desrosiers, B. Fleckenstein, C. Lopez, A. C. Minson, and M. J. Studdert.** 1992. The family *Herpesviridae*: an update. Arch. Virol. **123:** 425–449.
79. **Roizman, B., and N. Markovitz.** 1997. Herpes simplex virus virulence: the functions of the γ1 34.5 gene. J. Neurovirol. **3**(Suppl. 1)**:**S1–S2.
80. **Ros, C., and S. Belák.** 2002. Characterization of the glycoprotein B gene from ruminant alphaherpesviruses. Virus Genes **24:**99–105.
81. **Salzberg, S. L., A. L. Delcher, S. Kasif, and O. White.** 1998. Microbial gene identification using interpolated Markov models. Nucleic Acids Res. **26:**544–548.
82. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
83. **Schang, L. M., A. Hossain, and C. Jones.** 1996. The latency-related gene of bovine herpesvirus 1 encodes a product which inhibits cell cycle progression. J. Virol. **70:**3807–3814.
84. **Schwyzer, M., C. Vlcek, O. Menekse, C. Fraefel, and V. Paces.** 1993. Promoter, spliced leader, and coding sequence for BICP4, the largest of the immediate-early proteins of bovine herpesvirus 1. Virology **197:**349–357.
85. **Schwyzer, M., U. V. Wirth, B. Vogt, and C. Fraefel.** 1994. BICP22 of bovine herpesvirus 1 is encoded by a spliced 1.7 kb RNA which exhibits immediate early and late transcription kinetics. J. Gen. Virol. **75:**1703–1711.
86. **Smith, G. A., P. L. Young, and J. S. Mattick.** 1991. Nucleotide and amino acid sequence analysis of the thymidine kinase gene of a bovine encephalitis herpesvirus. Arch. Virol. **119:**199–210.
87. **Sonnhammer, E. L. L., S. R. Eddy, E. Birney, A. Bateman, and R. Durbin.** 1998. Pfam: multiple sequence alignments and HMM-profiles of protein domains. Nucleic Acids Res. **26:**320–322.
88. **Staden, A., and A. D. McLachlan.** 1982. Codon preference and its use in identifying protein coding regions in long DNA sequences. Nucleic Acids Res. **10:**141–156.
89. **Telford, E. A. R., M. S. Watson, K. McBride, and A. J. Davison.** 1992. The DNA sequence of equine herpesvirus-1. Virology **189:**304–316.
90. **Van Sant, C., Y. Kawaguchi, and B. Roizman.** 1999. A single amino acid substitution in the cyclin D binding domain of the infected cell protein 0 abrogates the neuroinvasiveness of herpes simplex virus without affecting its ability to replicate. Proc. Natl. Acad. Sci. USA **96:**8184–8189.
91. **Vogel, F. S. F., I. Oldoni, E. F. Flores, R. Weiblen, S. V. Mayer, and N. Winkelmann.** 2002. Investigation of neural and non-neural sites of latency by bovine herpesvirus type 5 in experimentally infected calves. Virus Rev. Res. **7:**63.
92. **Wallace, J. C., and S. Henikoff.** 1991. PATMAT: a searching and extraction program for sequence, pattern, and block queries and databases. Comput. Appl. Biol. Sci. **8:**249–254.
93. **Whetstone, C. A., B. S. Seal, and J. M. Miller.** 1993. Variability occurs in the inverted repeat region of genomic DNA from bovine herpesvirus 1 respiratory, genital and bovine herpesvirus 5 encephalitic isolates. Vet. Microbiol. **38:**181–189.
94. **Wirth, U. V., B. Vogt, and M. Schwyzer.** 1991. The three major immediate-early transcripts of bovine herpesvirus 1 arise from two divergent and spliced transcription units. J. Virol. **65:**195–205.
95. **Wirth, U. V., C. Fraefel, B. Vogt, C. Vlcek, V. Paces, and M. Schwyzer.** 1992. Immediate-early RNA 2.9 and early RNA 2.6 of bovine herpesvirus 1 are 3′ coterminal and encode a putative zinc finger transactivation protein. J. Virol. **66:**2763–2772.
96. **Wirth, U. V.** 1993. Comparison of immediate-early transcripts among bovine herpesvirus type 1 and type 5 strains differing in neurovirulent potential. Virus Res. **27:**1–12.