

# The Genome of Turkey Herpesvirus

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**Here we present the first complete genomic sequence of Marek's disease virus serotype 3 (MDV3), also known as turkey herpesvirus (HVT). The 159,160-bp genome encodes an estimated 99 putative proteins and resembles alphaherpesviruses in genomic organization and gene content. HVT is very similar to MDV1 and MDV2 within the unique long (UL) and unique short (US) genomic regions, where homologous genes share a high degree of colinearity and their proteins share a high level of amino acid identity. Within the UL region, HVT contains 57 genes with homologues found in herpes simplex virus type 1 (HSV-1), six genes with homologues found only in MDV, and two genes (HVT068 and HVT070 genes) which are unique to HVT. The HVT US region is 2.2 kb shorter than that of MDV1 (Md5 strain) due to the absence of an MDV093 (SORF4) homologue and to differences at the UL/short repeat (RS) boundary. HVT lacks a homologue of MDV087, a protein encoded at the UL/RS boundary of MDV1 (Md5), and it contains two homologues of MDV096 (glycoprotein E) in the RS. HVT RS are 1,039 bp longer than those in MDV1, and with the exception of an ICP4 gene homologue, the gene content is different from that of MDV1. Six unique genes, including a homologue of the antiapoptotic gene *Bcl-2*, are found in the RS. This is the first reported *Bcl-2* homologue in an alphaherpesvirus. HVT long repeats (RL) are 7,407 bp shorter than those in MDV1 and do not contain homologues of MDV1 genes with functions involving virulence, oncogenicity, and immune evasion. HVT lacks homologues of MDV1 oncoprotein MEQ, Cx36 chemokine, oncogenicity-associated phosphoprotein pp24, and conserved domains of phosphoprotein pp38. These significant genomic differences in and adjacent to RS and RL regions likely account for the differences in host range, virulence, and oncogenicity between nonpathogenic HVT and highly pathogenic MDV1.**

Turkey herpesvirus (HVT) is a ubiquitous, nonpathogenic virus of domestic turkeys (15, 78–80), and it is classified as the third serotype within the Marek's disease virus (MDV) group of antigenically and genetically related lymphotropic avian herpesviruses (15). This group also includes MDV serotype 1 (MDV1), which is the etiologic agent of the globally and economically significant Marek's disease in chickens (15). MDV1 is pathogenic in chickens, causing cytolitic infection in B cells, latent infection in T cells, and induction of T-cell lymphoma. MDV2 is nonpathogenic or of low pathogenicity in chickens (15).

HVT is nonpathogenic in chickens, but it does induce a viremia which is associated with induction of protective immune responses against MDV1 (15). Chickens infected with HVT become persistently infected and maintain long-lasting immunity (15, 29, 44, 53, 80). HVT appears to replicate less efficiently in skin than does MDV1, a phenotype that may be involved in the relatively infrequent transmission of HVT among chickens compared to attenuated strains of MDV1 and MDV2 (15–17).

HVT and combinations of HVT and nonpathogenic strains of MDV1 and MDV2 have been used extensively and effectively as vaccines against virulent MDV1 since the early 1970s (15, 44, 53, 80). Other benefits of HVT vaccines include the ability to genetically modify the virus for expression of protective heterologous antigens and the potential for in ovo vaccination. HVT vectors expressing genes from Newcastle disease

virus, infectious bursal disease virus, and infectious bronchitis virus confer protective systemic immune responses against these pathogens (19, 54, 58, 86). In ovo vaccination of late-stage (16- to 18-day) embryos with HVT leaves newly hatched chickens immune to challenge with pathogenic MDV1 (62, 63).

Although current vaccination strategies will effectively protect against most MDV1 isolates, they are not completely effective against newly emerging MDV1 strains of greater virulence (77). These strains which pose a constant threat to the poultry industry, are characterized by higher cytolitic activity, unusual tissue tropism, increased atrophy of lymphoid organs, immunosuppression, enhanced capacity to transform T cells, and earlier host death (77). The genetic basis underlying differences in viral virulence, oncogenicity, and host range among MDV strains is poorly understood.

Comparative genomics has proven useful in identifying genes with functions involving virulence and host range. The genomes of two MDV1 strains (GA and Md5) have been sequenced (45, 74), and the unique long and unique short (UL and US, respectively) regions of MDV2 are available (37). However, less than 30% of the HVT genome has been sequenced (5, 41, 43, 48, 68, 83, 85). Here we present the complete sequence of HVT strain FC-126 (Burmenester), with analysis and comparison to MDV1 and MDV2.

## MATERIALS AND METHODS

**DNA isolation, cloning, and sequencing.** HVT strain FC-126 (Burmenester), originally isolated from the blood of an asymptomatic turkey (79), was obtained from the American Type Culture Collection (Manassas, Va.). The virus was propagated in primary chicken embryo fibroblast cell culture, and viral DNA was extracted from the cytoplasm of infected cells as previously described (76). Random DNA fragments were obtained by incomplete enzymatic digestion with

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endonucleases *TaqI* and *AclI* (New England Biolabs, Beverly, Mass.). DNA fragments of 1.5 to 3 kbp were isolated after separation on agarose gels, cloned into the dephosphorylated *AclI* 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, using dideoxy-chain terminator sequencing chemistries (60) and an Applied Biosystem PRISM 377 automated DNA sequencer (PE Biosystems, Foster City, Calif.). ABI sequencing analysis software (version 3.3) was used for lane tracking and trace extraction. Bases were called from chromatogram traces with Phred (27), which also produced a quality file containing a predicted probability of error at each base position.

**DNA sequence analysis.** DNA sequences were assembled with Phrap (26), using the quality files and default settings to produce a consensus sequence which was manually edited with Consed (33). An identical sequence was assembled using the TIGR assembler with quality files and clone length constraints (72). 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 5,074 usable traces were assembled into a 140,525-bp contig by bidirectional sequencing of random clones and 28 PCR products. The assembled contig had an estimated error rate of <0.03% and no evidence of polymorphism using Polyphred analysis (26). Because the terminal and internal UL repeats (TRL and IRL, respectively) are identical and the internal and terminal US repeats (IRS and TRS, respectively) are identical, the coverage (redundancy at each base position) of the internal long and short repeats was approximately doubled. The assembled contig contained 663 bp of the TRS, all of the UL, IRL, IRS, and US, and 395 bp of the TRS (i.e., the full genome length minus the length of the TRL and IRL). TRL and TRS sequences were assembled separately with clones containing the TRL/UL and US/TRS junctions and overlapping clones, using clone length constraints and position as provided by the computer assembly programs. The TRL and IRL contigs were then joined to the main contig at the overlapping region in the TRL/UL and US/TRS boundaries, thus providing the complete genome. The predicted restriction map for HVT matched previously published data (13). For descriptive purposes, we have presented HVT in a linearized fashion as described by Dolan et al. (24). Genome DNA composition, structure, repeats, and restriction enzyme patterns were analyzed as previously described (2). Open reading frames (ORFs) encoding proteins of  $\geq 60$  amino acids with a methionine start codon (70, 71) were evaluated for coding potential using the Hexamer (ftp.sanger.ac.uk/pub/rd), Framefinder (www.hgmp.mrc.ac.uk/~gslater/ESTate/), and Glimmer (59) computer programs. Other criteria included similarity to other herpesvirus or cellular proteins and compact gene arrangement with little gene overlap (21, 73). Homology searches were conducted using BLAST (3), PSIBLAST (4), FASTA (52), BLIMPS (75), and HMMER (69) programs with the Prosite, Pfam, Prodom, Sbase, Blocks, Domo, and GenBank databases (14). GCG (22), MEMSAT (40), and SAPS (10) programs were used for gene analysis.

**Nucleotide sequence accession number.** The HVT genome sequence has been deposited in GenBank under accession no. AF291866.

## RESULTS AND DISCUSSION

**Genome organization.** The HVT genome is 159,160 bp long and contains a 47.5% G+C base composition. HVT is organized in the same overall manner as are other alphaherpesviruses (55) (Fig. 1). UL and US regions are 111,868 and 8,617 bp in length, respectively. Each unique region is bounded by identical inverted repeats. The TRL and IRL are 5,658 bp, and the IRS and TRS are 13,303 bp. As with other herpesviruses, the G+C content in the repeat regions is higher than in unique regions (55% in repeats versus 45% in unique regions) (21, 32, 73). HVT contains no retrovirus long terminal repeat sequences as have been described for cell culture-adapted MDV1 strains (36, 38, 39, 45). The assembled HVT sequence contains a 251-bp sequence that is identical at each terminus and at the IRL/IRS junction. This repeat contains sequences similar to alphaherpesvirus  $\alpha$ -type sequences (42) and includes 17 copies of the GGGTTA motif found in MDV1 (64 copies), human herpesvirus 6, and eukaryotic telomers (42, 74). In

addition, a 266-bp identical inverted repeat is located adjacent to the TRL at positions 5910 to 6175 and near the IRL at positions 116856 to 117121 (Fig. 1). A 656-bp unique sequence (117122 to 117177) separates the second 266-bp repeat from the IRL (Fig. 1).

**Gene characterization.** HVT contains 397 ORFs of 60 to 2,164 codons, of which 99 are estimated to be functional genes (Fig. 2). Seventy-five genes are present as single copies and initiate within unique regions. Two genes initiate within the US region but are partially located within repeat regions (HVT083 and HVT092). Twenty-two genes initiate and are completely located within repeat regions. HVT gene products are most similar to homologues from MDV1 (36 to 82% amino acid identity) and MDV2 (34 to 81% amino acid identity). Among nonavian herpesviruses, ORFs of equine herpesviruses 1 and 4 are most similar to those of HVT (27 to 62% amino acid identity).

HVT and MDV1 share 76 conserved gene homologues, 92% of which are within the UL and US regions. Only a single MDV1 gene homologue is present in the repeats. HVT contains 13 genes for which there are no MDV1 homologues, and it lacks homologues of 16 genes present in MDV1.

**UL region.** The UL region, extending from nucleotide positions 5910 to 117777, contains 67 probable genes (Fig. 1 and 2). HVT008 to HVT063 represent 62% of the HVT genome and are colinear with genes UL1 to UL54 of herpes simplex virus type 1 (HSV-1) and MDV013 to MDV068 of MDV1 (74). Predicted proteins are 37 to 82% identical to MDV1 homologues. Those with the greatest (>70%) amino acid identity to MDV1 proteins correspond to capsid proteins (HVT025 and HVT026), viral replication proteins (HVT012, HVT016, HVT022, HVT037, HVT038, HVT039, HVT047, and HVT048), membrane proteins (HVT035, HVT052, and HVT053), and phosphoproteins (HVT010 and HVT061). These proteins are also the most conserved in comparison with MDV2 and other nonavian herpesviruses. HVT tegument proteins are the least conserved within the UL, with seven (HVT018, HVT021, HVT028, HVT044, HVT054, HVT055, and HVT057) sharing less than 51% amino acid identity with MDV1 homologues. Other, less conserved UL proteins include homologues of HSV virion surface glycoprotein L (HVT008), glycoprotein K (HVT062), and proteins of unknown function (HVT030, HVT051, HVT064, HVT065, and HVT066). HVT044 (large tegument protein) is 230 amino acids shorter than its MDV1 homologue due to the lack of a highly repetitive central proline-rich domain.

Six predicted HVT genes located at the ends of the UL region are MDV specific. The absence of HVT006 (MDV011), HVT007 (MDV012), HVT064 and HVT067 (MDV069), HVT069 (MDV072), and HVT071 (MDV073) (Fig. 2) in non-MDV alphaherpesviruses suggests an avian host range function, perhaps involving some aspects of lymphotropism. On the left end, HVT lacks homologues of MDV008 (pp24) and MDV009, a gene of unknown function (74). On the right end, HVT contains two novel genes (HVT068 and HVT070) which are absent in MDV1 and encodes two homologues (HVT064 and HVT067) of MDV069.

HVT005 is similar to eukaryotic and viral lipases (GenBank accession no. AF007578 and L43561) and shares 41 to 45% identity with MDV homologues (6, 74). HVT005 contains the

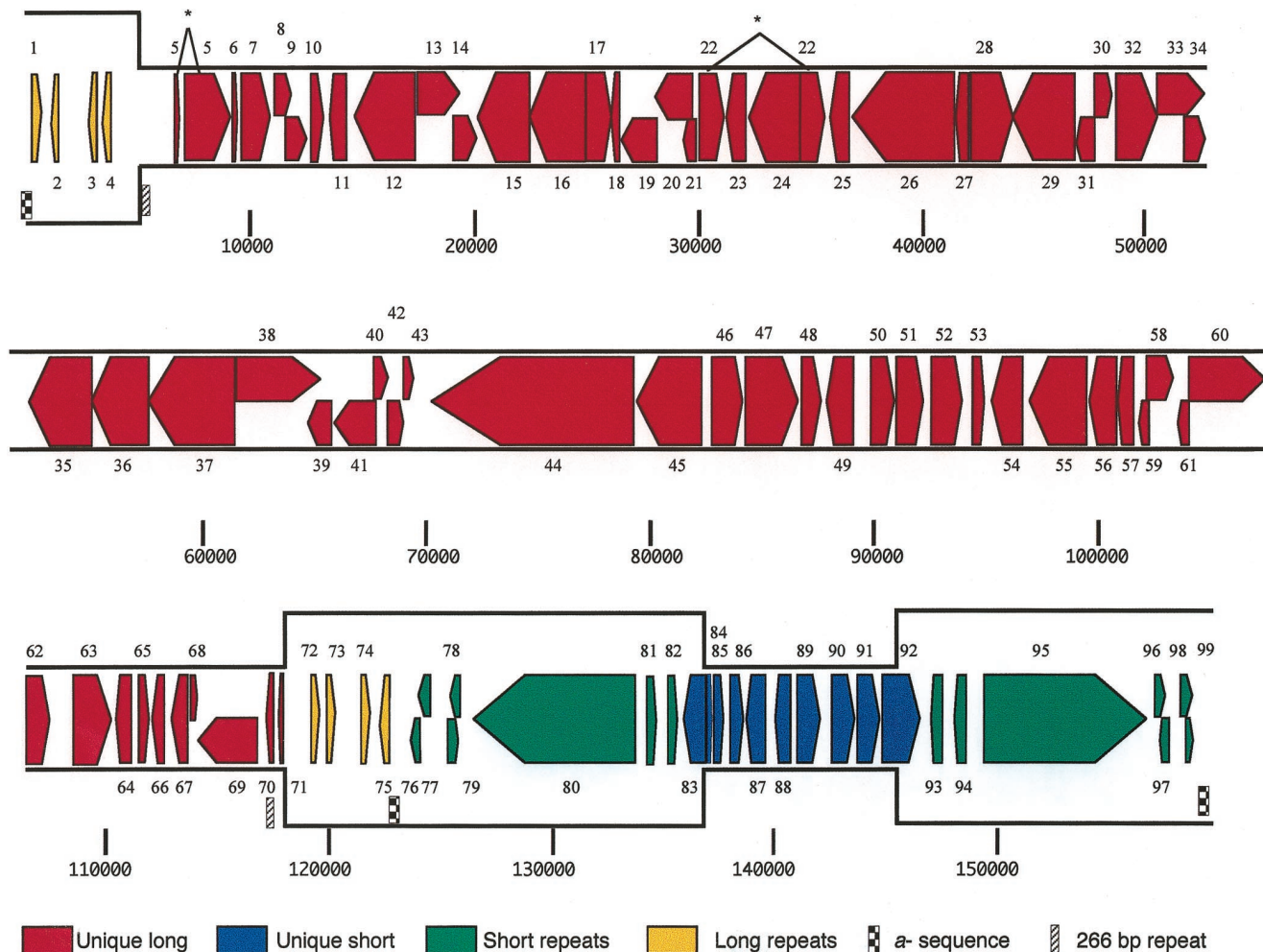


FIG. 1. Linear map of the HVT genome. Genes (colored arrows) are numbered from left to right based on positions of methionine initiation codons and transcribed in the direction indicated. Genes included in different genomic regions are defined by the color key. Nucleotide positions are indicated below the map. Spliced genes are indicated by asterisks.

serine active site at amino acid position 287 within the lipase signature motif (IxIGHxSxS, Prosite PS00120) and conserved cysteines involved in disulfide bond formation (amino acid positions 387 and 397). The region between amino acids 120 and 265 is similar to corresponding regions in eukaryotic lipases such as phospholipase A1 (26% over 154 amino acids). Similarity to predicted proteins from MDV1, MDV2, and fowl adenovirus extends beyond this region, suggesting the presence of additional virus-specific domains. The presence of a signal peptide in the amino-terminal domain and a transmembrane domain at amino acid positions 479 to 501 suggests that HVT005 may be membrane localized. HVT005 may perform host range functions involving alteration of host lipid metabolism and/or modification of second-messenger signaling pathways (1, 23, 31, 35, 50, 61, 66). Altered lipid metabolism has been observed both in vivo and in cell cultures during MDV infection (28, 34).

**US region.** The US region, extending from positions 136990 to 145606, has been previously sequenced (83). This region encodes 10 likely genes (HVT083 to HVT092), of which 7 are

homologues of the HSV-1 genes US1, US2, US3, US6, US7, US8, and US10. Proteins encoded in the HVT US region are 39 to 66% identical to their homologues in MDV1 and overall are less conserved (48%) than those in the UL region (61%). The arrangement of genes in the HVT US region is similar to that in MDV1 and MDV2 (37, 74), including the inversion-translocation of the UL10 homologue, compared to HSV-1 (11, 21, 49, 56, 73, 87). HVT087, a homologue of MDV090, is found only in MDV. In addition, an HVT-specific gene of unknown function (HVT084) is found near the IRS/US boundary. HVT lacks a homologue of MDV093 (SORF4), a gene found in MDV1.

The US short repeat (RS) junction region is variable among MDV1, MDV2, and HVT (11, 37, 74, 83). Unlike MDV1 and MDV2, the carboxyl terminus of the HVT US8 gene homologue (glycoprotein E [gE] gene) is duplicated and inverted to the other end of the US. The presence of two copies of the gE ORF does not imply that the virus expresses two forms of gE. Thus, the HVT US/RS boundaries are located within the US8 homologues (HVT083 and HVT092 genes) and reduce the size

ORF	Position (nt) (length [aa])	HVT Accession no.	Md5 gene	MDV1 Blast score	MDV2 Length (aa) (% Identity)	Accession no.	Blast score	Name	Closest Accession no. b	non-MDV Blast score	species length (aa) (% Identity)	HSV-1 homologue	Predicted structure and/or function
HVT001	264-767(168)		MDV010	1561	666 (45%)	AB024414	1517	742(41%)	AActv8	260	325 (26%)		Lipase
HVT002	1618-1292(109)		MDV011	137	74(45%)	AB024414	535	379(36%)		119	104 (26%)	UL1	Viron surface glycoprotein L
HVT003	3618-3412(69)		MDV012	494	393(36%)	AB024414	458	180(48%)		699	273 (52%)	UL2	Uracil-DNA glycosylase
HVT004	5244-5038(69)		MDV013	463	176(48%)	AB024414	704	208(68%)		551	207 (57%)	UL3	Nuclear phosphoprotein
HVT005	6605-6701(728)		MDV014	943	313(58%)	AB024414	611	243(53%)		227	141 (38%)	UL4	Nuclear protein
	6777-8863		MDV015	717	210(70%)	AB024414	3390	848(75%)		2760	850 (61%)	UL5	DNA helicase-primase associated protein
			MDV016	667	241(58%)	AB024414	2157	710(59%)		1444	699 (44%)	UL6	Minor capsid protein, DNA packaging
			MDV017	3541	858(79%)	AB024414	2819	875(63%)		1026	741 (34%)	UL7	DNA helicase-primase associated protein
			MDV018	2243	714(61%)	AB024414	2280	757(54%)		2041	836 (50%)	UL8	Orf-binding protein
			MDV019	895	305(58%)	AB024414	2819	875(63%)		583	415 (33%)	UL9	Viron membrane glycoprotein M
			MDV020	2305	750(56%)	AB024414	1580	414(70%)		74	44 (39%)	UL10	Myristylated tegument protein
			MDV021	3129	841(70%)	AB024414	133	78(42%)		825	488 (40%)	UL11	DNAse
			MDV022	1568	424(69%)	AB024414	1364	473(56%)		910	448 (42%)	UL12	Serine/threonine protein kinase, tegument
			MDV023	180	77(49%)	AB024414	1639	471(66%)		255	201 (33%)	UL13	Minor tegument protein
			MDV024	1404	508(54%)	AB024414	2911	733(75%)		2295	735 (62%)	UL14	DNA packaging protein (terminase)
			MDV025	1604	436(68%)	AB024414	1040	350(54%)		520	327 (36%)	UL16	Tegument protein
			MDV026	545	238(49%)	AB024414	1900	726(52%)		851	724 (33%)	UL17	Tegument protein, DNA packaging
			MDV027	3004	737(77%)	AB024414	1292	321(73%)		770	308 (47%)	UL18	Capsid protein
			MDV028	1108	344(59%)	AB024414	6103	1389(80%)		4213	1359 (58%)	UL19	Major capsid protein
			MDV029	1916	726(53%)	AB024414	566	227(49%)		248	196 (32%)	UL20	Membrane protein, virus egress
			MDV030	1296	319(74%)	AB024414	1214	532(48%)		531	330 (40%)	UL21	Tegument protein
			MDV031	6237	1390(82%)	AB024414	2089	779(51%)		592	621 (27%)	UL22	Envelope glycoprotein H
			MDV032	575	234(50%)	AB024414	515	209(52%)		354	178 (42%)	UL23	Thymidine kinase
			MDV033	1315	545(48%)	AB024414	1018	333(57%)		469	338 (34%)	UL24	DNA packaging
			MDV034	2358	806(56%)	AB024414	1964	581(65%)		1283	584 (48%)	UL25	Capsid maturation protease
			MDV035	504	265(46%)	AB024414	1427	638(50%)		794	635 (37%)	UL26.5	Capsid maturation protease
			MDV036	1030	342(58%)	AB024414	3508	855(76%)		2409	834 (53%)	UL27	minor capsid scaffold protein
			MDV037	1994	591(63%)	AB024414	2745	785(66%)		1785	758 (47%)	UL28	DNA packaging protein
			MDV038	1563	663(53%)	AB024414	4347	1200(69%)		3302	1205 (55%)	UL29	Single stranded DNA binding protein
			MDV039	1563	663(53%)	AB024414	1150	305(70%)		853	304 (55%)	UL30	DNA polymerase catalytic subunit
			MDV040	3748	865(81%)	AB024414	2003	135(60%)		261	118 (49%)	UL31	Nuclear phosphoprotein
			MDV041	2890	787(68%)	AB024414	403	623(64%)		1258	630 (45%)	UL32	Role in DNA packaging
			MDV042	5029	1191(78%)	AB024414	876	276(63%)		491	254 (45%)	UL33	Role in DNA packaging
			MDV043	4607	1219(71%)	AB024414	2003	135(60%)		261	118 (49%)	UL34	Membrane phosphoprotein
			MDV044	1135	305(70%)	AB024414	353	126(57%)		127	98 (32%)	UL35	Capsid protein
			MDV045	437	130(63%)	AB024414	7148	3105(50%)		2865	2437 (31%)	UL36	Large tegument protein
			MDV046	2180	641(66%)	AB024414	2982	1025(56%)		1292	1025 (30%)	UL37	Tegument protein
			MDV047	916	273(64%)	AB024414	1448	468(60%)		853	380 (48%)	UL38	Capsid protein
			MDV048	394	127(63%)	AB024414	3078	787(74%)		1992	744 (55%)	UL39	Ribonucleotide reductase, large subunit
			MDV049	7398	3282(50%)	AB024414	1288	302(81%)		1033	331 (60%)	UL40	Ribonucleotide reductase, small subunit
			MDV050	3208	1043(59%)	AB024414	1817	484(68%)		805	445 (40%)	UL41	Viron host shut-off protein, tegument
			MDV051	1488	469(61%)	AB024414	859	369(64%)		342	282 (31%)	UL42	DNA polymerase processivity subunit
			MDV052	3345	818(78%)	AB024414	1292	369(64%)		166	368 (24%)	UL43	Probable membrane protein
			MDV053	1305	312(78%)	AB024414	1817	484(68%)		362	286 (30%)	UL44	Viron membrane glycoprotein C
			MDV054	1441	433(64%)	AB024414	859	369(64%)		119	166 (28%)	UL45	Envelope/membrane protein, cell fusion
			MDV055	1407	369(69%)	AB012572	1292	369(64%)		381	406 (29%)	UL46	Tegument phosphoprotein
			MDV056	1068	422(50%)	AB024414	1817	484(68%)		281	464 (24%)	UL47	Tegument phosphoprotein
			MDV057	1999	488(75%)	AB024414	859	369(64%)		785	405 (41%)	UL48	Immediate early gene transactivator, tegument
			MDV058	861	212(73%)	AB024414	729	212(65%)		234	176 (33%)	UL49	Tegument phosphoprotein
			MDV059	793	520(37%)	AB024414	700	334(42%)		318	160 (41%)	UL50	dUTPase
			MDV060	1731	793(46%)	AB024414	1486	806(44%)					
			MDV061	1211	422(55%)	AB012572	957	400(48%)					
			MDV062	449	243(42%)	AB024414	450	238(42%)					
			MDV063	1517	435(64%)	AB024414	1243	390(59%)					



HVT059	102435-102154(94)	MDV064	281	95(61%)	AB024414	267	95(60%)	BHV-1	S62816	97	59 (35%)	UL49.5	Envelope/tegument protein
HVT060	104530-107763(1078)	MDV066	3499	1071(62%)	AB024414	3391	1066(59%)	EHV-4	AF030027	1918	1069 (41%)	UL52	DNA helicase-primase associated protein
HVT061	104531-103812(240)	MDV065	634	162(76%)	AB024414	589	159(72%)	VZV	P09271	351	149 (47%)	UL51	Virion phosphoprotein
HVT062	107745-108791(349)	MDV067	877	354(48%)	AB024414	776	354(43%)	VZV	P09261	430	333 (30%)	UL53	Glycoprotein K
HVT063	108952-110379(476)	MDV068	1176	475(54%)	AB024414	943	475(48%)	EHV-1	Q05906	428	469 (29%)	UL54	Post-translational gene regulation
HVT064	111303-110506(266)	MDV069	705	256(50%)	AB024414	455	181(46%)	EHV-1	P28963	195	158 (34%)	UL55	VZV <i>orf-2</i> , EHV-4 gene 3
HVT065	111500-112006(169)	MDV070	424	164(49%)	AB024414	386	161(47%)	EHV-4	AF030027	151	171 (27%)		
HVT066	112765-112211(185)	MDV071	337	143(46%)	AB024414	450	182(47%)						
HVT067	113905-113111(265)	MDV069	742	259(54%)	AB024414	450	182(47%)						
HVT068	113922-114170(83)	MDV072	1653	873(43%)	AB024414	1559	870(41%)						
HVT069	116730-114082(883)	MDV073	115	72 (38%)	AB024414	114	79(34%)						
HVT070	117221-116835(129)												
HVT071	117574-117323 (84)	Z46371											Truncated pp38
HVT072	118443-118649(69)												
HVT073	120069-120275(69)												
HVT074	122069-122395(109)												
HVT075	123423-122920(168)												
HVT076	123898-123521(126)												
HVT077	124251-123709(181)												
HVT078	124918-125463(182)												
HVT079	125510-125025(162)												
HVT080	133136-126645(2164)												
HVT081	133768-134010(81)	MDV084	3592	2302(40%)				EHV-1	Japanese quail X64418	479	146 (66%)	RS1	Apoptosis inhibitor, <i>Bcl-2</i> family
HVT082	135146-135373(76)								P17473	1091	1230 (33%)		Immediate-early gene transactivator, ICP4
HVT083	137052-136048(335)	MDV096	701	311(44%)	AB016432	528	294(40%)	FHV-1	S72415	238	311 (26%)	US8	Membrane glycoprotein E
HVT084	137055-137282(76)	MDV088	237	120(39%)	AB016432	234	84(50%)	FHV-1	D42113	161	100 (34%)	US1	Immediate-early phosphoprotein, ICP22
HVT085	137326-137820(165)	MDV089	420	185(42%)	AB016432	411	190(41%)	EHV-1	P28965	217	180 (34%)	US10	Virion protein
HVT086	138058-138684(209)	MDV090	757	345(46%)	AB016432	659	267(46%)	ILT	U28832	79	102 (27%)	MDV1 SORF3	
HVT087	139866-138829(346)	MDV091	908	259(66%)	AB016432	863	251(62%)	CHV	U84223	285	173 (35%)	US2	Serine/threonine protein kinase
HVT088	140913-140068(282)	MDV092	1201	386(59%)	AB016432	1101	385(57%)	CHV	U84223	563	364 (38%)	US3	Membrane glycoprotein D
HVT089	141063-142244(394)	MDV093	830	347(48%)	AB016432	761	376(41%)	BHV-1	A25177	247	224 (30%)	US6	Membrane glycoprotein I
HVT090	142588-143739(384)	MDV094	610	334(40%)	AB016432	485	348(35%)	CHV	U84223	246	320 (26%)	US7	Membrane glycoprotein E
HVT091	143832-144899(356)	MDV095	1121	493(44%)	AB016432	875	477(39%)	FHV-1	S72415	378	498 (26%)	US8	
HVT092	145055-146548(498)	MDV096	1121	493(44%)	AB016432	875	477(39%)	FHV-1					
HVT093	147450-147223(76)												
HVT094	148828-148586(81)	MDV084	3592	2302(40%)				EHV-1	P17473	1091	1230 (33%)	RS1	Immediate-early gene transactivator, ICP4
HVT095	149460-155951(2164)							Japanese quail	X64418	479	146 (66%)		Apoptosis inhibitor, <i>Bcl-2</i> family
HVT096	157086-157571(162)												
HVT097	157678-157133(182)												
HVT098	158345-158887(181)												
HVT099	158698-159075(126)												

FIG. 2. Accession numbers are from GenBank, SwissProt, and PIR databases; the accession number for Md5 genes is AF243438. Names for viruses: bovine herpesvirus (BHV), canine herpesvirus (CHV), duck enteritis virus (DEV), equine herpesviruses 1 and 4 (EHV-1 and -4), feline herpesvirus (FHV), herpes simplex virus (HSV), herpesvirus of turkeys (HVT), infectious laryngotracheitis virus (ILT), Marek's disease virus serotypes 1 and 2 (MDV1 and -2), varicella-zoster virus (VZV), ceropithecine herpesvirus 9 (CPHV), and avian adenovirus type 8 (Aadv8). Function was deduced either from the degree of amino acid (aa) similarity to known genes or by the presence of Prosite signatures.



FIG. 3. Multiple amino acid sequence alignment of HVT096 with Bcl-2 family members. Asterisks mark anti- and proapoptotic domains, and shaded residues indicate identity to HVT096. Gaps generated during the alignment with Pileup are indicated with dashes. Amino acid positions are indicated on the right. Rat, *Rattus norvegicus*, accession no. U34963; Chicken, *Gallus gallus*, accession no. Q07816; Quail, *Coturnix japonica*, accession no. X84418; BHV-4, bovine herpesvirus 4, accession no. AF129421.

of the US compared to MDV1 and MDV2. Duplication of US genes and resulting changes in US/RS boundary position has been noted between strains of MDV1 and with other herpesviruses (11, 20, 74). Like MDV2, HVT lacks a homologue of the MDV087 (SORF2) gene. SORF2 is nonessential for MDV1 replication in cell culture, but it is duplicated in the very virulent MDV1 strain Md5 (11, 51, 74). Although the function of this gene is unknown, its presence in fowlpox virus and fowl adenovirus suggests a significant avian host range function (2, 65).

**RL.** The RL are 5,658 bp in length and are located at nucleotide positions 252 to 5909 and 117778 to 123435. HVT RL are 7,407 bp shorter than MDV1 RL and lack homologues of all MDV1 RL genes. Each RL contains four possible HVT-specific genes, which predict proteins of unknown function (HVT001 to HVT004 and HVT072 to HVT075). Although there are no other readily identifiable genes in this region based on our criteria, the RL does contain 21 ORFs of more than 60 codons. Thus, additional genes may be present.

Most notably, and consistent with its nonpathogenic phenotype, HVT lacks homologues of all MDV1 RL proteins with putative functions involving viral virulence, host range, and oncogenicity. HVT contains no homologues of the Marek's *EcoRI* Q protein (MEQ) (MDV005 and MDV076), which is associated with cellular transformation and oncogenicity (45, 74). In addition, HVT lacks pp24 (MDV008) and conserved domains of pp38 (MDV073), phosphoproteins associated with cellular transformation (18, 81, 88). The functional homology of HVT071 to pp38 is unclear. HVT071 lacks the amino terminus (75 amino acids) and a centrally located (22-amino-acid) conserved region containing a DLLVEAE motif found in pp38 and pp24 (74). Moreover, recent experiments have shown that deletion of this putative gene from the HVT genome has no effect on viral growth or virulence (12). HVT lacks a homologue of the MDV1 CxC chemokine (MDV003 and MDV078) (74) and the 132-bp repeat region whose expansion has been

associated with altered viral transcription and loss of oncogenicity (7, 9, 30, 47, 57, 67).

**RS.** The short repeat regions (RS) are 13,303 bp and are located at nucleotide positions 123687 to 136989 and 145607 to 158909. Each RS encode seven possible genes, of which five lack homology to other known genes (Fig. 1 and 2). HVT080 and HVT095 encode homologues of the HSV-1 major immediate-early *trans*-activating protein ICP4. These genes, which encode proteins of 2,164 amino acids, comprise over 48% of the RS. Interestingly, HVT080 and HVT095 contain an 810-amino-acid amino-terminal extension which is absent in ICP4 homologues of other nonavian alphaherpesviruses. A similarly sized ICP4 homologue has been previously found in MDV1 and predicted to exist in HVT (74, 84). HVT lacks identifiable homologues of immediate-early proteins ICP0, which is non-essential for replication of HSV-1 in cell culture (55), and ICP47, a host range protein which blocks HSV-1 antigen presentation on infected cells (82). HVT also lacks identifiable homologues of MDV085, a protein of unknown function, and MDV086, a gene present in a region transcribed during viral latency (74).

HVT079 and HVT096 encode identical 162-amino-acid proteins with significant similarity (66% amino acid identity) to the quail Nr13 protein, a homologue of Bcl-2 (Fig. 3) (46). Amino acid identity occurs over the entire length of the protein and includes Bcl-2 BH1, BH2, and BH3 domains and a novel form of the BH4 domain (46). HVT079 and HVT096 lack the 15-amino-acid carboxyl-terminal transmembrane domain present in Nr13 and in other mammalian Bcl-2 homologues. Although all sequenced gammaherpesvirus genomes encode Bcl-2 homologues (8), HVT079 and HVT096 represent the first Bcl-2 homologues found in an alphaherpesvirus.

HVT079 and HVT096 likely function in promoting infected cell survival and may account for differences in cell and tissue tropism (15, 62, 64). *Nr13*, a developmentally regulated gene which is maximally expressed at 15 to 21 days in the chicken

bursa of Fabricius, may play a role in maintaining bursal stem cell populations (46). Additionally, *in vitro* studies have shown the *Nr13* can protect bursal lymphoma cells from low-serum-induced apoptosis (46). Recent experimental evidence suggests that HVT, but not MDV1, contains genes capable of preventing cell death (25). Induction of apoptosis by serum deprivation in transformed chicken B-cell lines was prevented by infection with HVT but not by MDV1 (25).

**Conclusions.** HVT resembles other alphaherpesviruses in genome organization and gene content. A close relationship among MDV serotypes is evident by the high degree of amino acid identity of protein homologues and by the presence of MDV-specific proteins. Significant genomic differences occur between HVT and MDV1 in and adjacent to RL and RS, and these may account for differences in tissue tropism, host range, and the nonpathogenic phenotype of HVT (15). HVT lacks putative MDV1 virulence and host range genes, including MEQ, pp24, pp38, CxC chemokine, MDV087 (SORF2), and MDV093 (SORF4). There are 13 HVT-specific genes, which include a *Bcl-2* homologue. Thus, comparative analysis of nonpathogenic HVT and MDV2 and virulent MDV1 genomes enhances our overall understanding of MDV virulence and host range. This information will permit the engineering of novel MDV vaccine viruses and avian expression vectors with enhanced efficacy and greater versatility.

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