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The Genomes of Sheeppox and Goatpox Viruses

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Sheeppox virus (SPPV) and goatpox virus (GTPV), members of the *Capripoxvirus* genus of the *Poxviridae*, are etiologic agents of important diseases of sheep and goats in northern and central Africa, southwest and central Asia, and the Indian subcontinent. Here we report the genomic sequence and comparative analysis of five SPPV and GTPV isolates, including three pathogenic field isolates and two attenuated vaccine viruses. SPPV and GTPV genomes are approximately 150 kbp and are strikingly similar to each other, exhibiting 96% nucleotide identity over their entire length. Wild-type genomes share at least 147 putative genes, including conserved poxvirus replicative and structural genes and genes likely involved in virulence and host range. SPPV and GTPV genomes are very similar to that of lumpy skin disease virus (LSDV), sharing 97% nucleotide identity. All SPPV and GTPV genes are present in LSDV. Notably in both SPPV and GTPV genomes, nine LSDV genes with likely virulence and host range functions are disrupted, including a gene unique to LSDV (LSDV132) and genes similar to those coding for interleukin-1 receptor, myxoma virus M003.2 and M004.1 genes (two copies each), and vaccinia virus F11L, N2L, and K7L genes. The absence of these genes in SPPV and GTPV suggests a significant role for them in the bovine host range. SPPV and GTPV genomes contain specific nucleotide differences, suggesting they are phylogenetically distinct. Relatively few genomic changes in SPPV and GTPV vaccine viruses account for viral attenuation, because they contain 71 and 7 genomic changes compared to their respective field strains. Notable genetic changes include mutation or disruption of genes with predicted functions involving virulence and host range, including two ankyrin repeat proteins in SPPV and three kelch-like proteins in GTPV. These comparative genomic data indicate the close genetic relationship among capripoxviruses, and they suggest that SPPV and GTPV are distinct and likely derived from an LSDV-like ancestor.

Capripoxviruses (CaPVs) represent one of eight genera within the chordopoxvirus (ChPV) subfamily of the *Poxviridae*. The *Capripoxvirus* genus is currently comprised of sheeppox virus (SPPV), goatpox virus (GTPV), and lumpy skin disease virus (LSDV), causing disease in sheep, goats, or cattle, respectively. These viruses are responsible for some of the most economically significant diseases of domestic ruminants in Africa and Asia (13, 19).

Sheeppox and goatpox are endemic throughout southwest and central Asia, the Indian subcontinent, and northern and central Africa (13, 19). In contrast, LSDV occurs largely in central and southern Africa and is absent in Asia. Sheeppox and goatpox exhibit similar clinical signs that are typical of generalized poxviral diseases, including pyrexia, cutaneous lesions, and notably the development of lung lesions (19, 45). Transmission of sheeppox and goatpox is efficient and suspected to occur via aerosol and insect vector (19, 36, 38).

CaPVs are generally considered to be host specific, because disease outbreaks or virus isolates may preferentially occur or cause disease in one host species (19, 45). This has been shown specifically for Nigerian, Middle Eastern, and Indian strains of SPPV and GTPV and for LSDV (32, 34, 37, 46, 50, 51). However, the ability of SPPV and GTPV strains to naturally or experimentally cross-infect and cause disease in both host species has been described previously (16, 35, 37). This apparent variability in SPPV and GTPV host range, the clinical similarity between sheeppox and goatpox, and the inability to differentiate the two diseases by serological assays have led to the suggestion that sheeppox and goatpox are part of a disease complex caused by a single viral species and that observable host range specificities are the result of regional virus adaptations to sheep or goat hosts (17, 37).

Restriction endonuclease analysis and cross-hybridization studies of SPPV and GTPV indicate that these viruses, although closely related (estimated 96 to 97% nucleotide identity), can be distinguished from one another and may undergo recombination in nature (8, 25–27, 32). These data and limited SPPV and GTPV DNA sequence analysis also indicate a high degree of similarity to LSDV, whose genome sequence contains a conserved ChPV-like complement of replicative genes and a unique complement of virulence and host range genes (11, 23, 25, 26, 29, 59).

Live attenuated SPPV and subunit formulations have been used experimentally and in enzootic and outbreak areas as vaccines against sheeppox, goatpox, and lumpy skin disease (12–14, 33, 47). However, vaccine-induced disease, vaccine failure, and restrictions on the use of live virus vaccines in nonenzootic areas create the need for improved CaPV vaccines (13, 51, 61). An improved understanding of the genetic basis of CaPV virulence and host range will permit rational design of vaccines having greater efficacy and versatility.

Given the economic significance of CaPVs, their potential

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for spread into nonenzootic regions, and interest in developing more effective CaPV-based vaccines and expression vectors, we have sequenced and analyzed the genomes of three SPPVs and two GTPVs. These data, combined with LSDV sequence data, provide a comparative view of CaPV genomics and describe the genetic basis for CaPV virulence and host range.

MATERIALS AND METHODS

Virus strains. The following sequenced viruses were used: SPPV strain TU, isolated during an outbreak of sheeppox in Turkey in the late 1970s, passaged six times in lamb testicle (LT) cells and once in sheep coroid plexus cells, and subsequently reisolated in 2000 from lung lesions of an experimentally infected sheep (Plum Island Animal Disease Center, Animal and Plant Health Inspection Service, U.S. Department of Agriculture, Greenport, N.Y.); SPPV strain A (SA), a field isolate obtained from a sick sheep in the Almatinskaya region, Kazakhstan, and passaged nine times in sheep at the Scientific Research Agricultural Institute (SRAI), Kazakhstan (1987); SPPV strain Niskhi (NK), an attenuated vaccine strain derived from an epizootic strain through 30 passages in lamb kidney (LK) cell cultures at SRAI (1994); GTPV strain Pellor (PL), a pathogenic field isolate passaged three times in LK at SRAI (1996); and GTPV strain G_{20}-LKV (GV), a vaccine strain derived from a field isolate of low pathogenicity through 20 passages in LK at SRAI (2000).

Viral DNA isolation, cloning, sequencing, and sequence analysis. Viral genomic DNA was extracted from primary LT cells. Random DNA fragments were obtained by incomplete enzymatic digestion with *Tsp*509I endonuclease (New England Biolabs, Beverly, Mass.), and DNA fragments larger than 1.0 kbp were cloned and used in dideoxy sequencing reactions as previously described (3). Reaction products were analyzed on an ABI PRISM 3700 automated DNA sequencer (Applied Biosystems, Foster City, Calif.). Sequence data were assembled with the Phrap and CAP3 software programs (20, 30), and gaps were closed as described previously (2). The final DNA consensus sequences for each genome represented on average seven to ninefold redundancy at each base position and a Consed estimated error rate of 0.01 to 0.03 per 100 kbp (20, 21, 28).

Genome DNA composition, structure, repeats and restriction enzyme patterns were analyzed as previously described (2) with the Genetics Computer Group GCG v.10 software package (18). Pairwise genomic alignments were done with WABA (Jim Kent; http://www.cse.ucsc.edu/~kent/) and multiple genomic alignments were done with Dialign (42) and Clustal (58) alignment programs. Open reading frames (ORFs) longer than 30 codons were evaluated for coding potential as previously described (3). All ORFs with coding potential and ORFs greater than 60 codons were subjected to homology searches as previously described (2, 3). Based on these criteria, at least 147 ORFs were annotated as potential genes and numbered to coincide with orthologous ORFs from LSDV (59). Phylogenetic comparisons were done with the PHYLO_WIN software package (22).

Nucleotide sequence accession number. The genome sequences of SPPV strains TU, SA, and NK, and GTPV strains PL and GV have been deposited in GenBank under accession no. AY077832, AY077833, AY077834, AY077835, and AY077836, respectively.

RESULTS AND DISCUSSION

SPPV and GTPV genomes. Genome sequences of SPPV field isolate strains TU and SA, SPPV vaccine strain NK, GTPV field isolate PL, and GTPV vaccine strain GV were assembled into contiguous sequences of 149,955, 150,057, 149,662, 149,935, and 149,695 bp, respectively. This agrees with previous restriction enzyme-based size estimates for SPPV and GTPV (SGPV) genomes of approximately 143 to 147 kbp (25). Because poxvirus hairpin loop sequences known to be present at CaPV termini were not sequenced, the leftmost nucleotide of each assembled genome was arbitrarily designated base 1 (8, 43). Nucleotide composition is approximately 75% A+T uniformly distributed for all genomes.

SPPV and GTPV genomes, like those of other poxviruses, contain a central coding region bounded by two identical inverted terminal repeat (ITR) regions. Assembled ITRs of SPPV TU, SA, NK, and GTPV PL and GV contain at least 2,213, 2,349, 2,127, 2,305, and 2,198 bp, respectively, similar to those previously estimated for SPPV (25). Sequences similar to noncoding tandem repeats previously described in SPPV and LSDV terminal regions were identified in SPPV TU, SA, NK, and GTPV PL and GV ITRs (24, 59). Available data from terminal genomic regions indicate that CaPVs differ in the nature and size of tandemly repeated sequence. Notably, all SPPV isolates contain 46 bp of the ORFs closest to the termini (ORFs 001 and 156) in their tandem repeats, compared to LSDV, which contains 5 bp of ORFs 001 and 156 in its tandem repeats (24, 59). Although ORFs 001 and 156 were completely sequenced in each GTPV strain, no terminal tandem repetition was noted. Comparison with experimental and published restriction fragment analysis indicates that additional terminal repeats and hairpin loop sequences of less than 200 bp may be present in each genome (8, 25, 32) (data not shown).

SGPV field isolates contain 147 ORFs, which have been annotated here as putative genes and as orthologues of LSDV genes (Table 1). These genes represent an approximate 93% coding density and encode proteins of 53 to 2,027 amino acids (Table 1). The central genomic region of SGPV (ORFs 024 to 123) contains homologues of conserved poxvirus genes involved in basic replicative mechanisms, including viral transcription and RNA modification, viral DNA replication, and structure and assembly of intracellular mature and extracellular enveloped virions (43) (Table 1). Terminal genomic regions of SGPV (ORFs 001 to 0023 and ORFs 124 to 156) contain genes with putative virulence and host range functions similar to those found in LSDV (59). These include gene families (five genes with ankyrin repeat motifs and three genes with kelch-like repeat motifs) and other genes likely involved in viral modification or evasion of host cellular, apoptotic, and immune responses or processes, including homologues of cytokine binding proteins, interleukin-10 (IL-10), an epidermal growth factor (EGF)-like protein, PKR inhibitors, a serpin, and poxvirus-specific virulence and host range genes (Table 1) (5, 43).

Comparison of SPPV and GTPV. SPPV and GTPV are highly similar to each other at the genomic level, sharing colinearity (147 orthologous genes) and average nucleotide identity of 96% over the length of their genomes (Tables 1 and 2). Intraspecies nucleotide identity was greater than 99%, because SPPV TU and SA contained only 192 genomic changes, including 131 single-nucleotide substitutions (Table 2). Analysis of entire genome sequences suggests that SPPV and GTPV, although highly similar, are phylogenetically distinct (Fig. 1). This phylogenetic grouping of CaPVs isolated from a given host species supports a genetic basis for CaPV species-specific host range, and it agrees with data suggesting that viruses of sheep and goat origins can be differentiated via genomic restriction fragment pattern analysis (8, 25, 32).

SPPV and GTPV field isolates demonstrate average amino acid differences of 4% (Table 1). SPPV SA and GTPV PL share 101, 39, and 7 genes with 96 to 100%, 91 to 95%, and 80 to 90% amino acid identity, respectively (Table 1). Twenty-six SPPV and GPV ORFs differ in size (1 to 18 amino acids) due to insertion or deletion of amino acids within the ORF or alterations in start or stop codons. SPPV TU ORFs 003 and 154 are 73 amino acids longer than in other SPPVs due to two

TABLE 1. SPPV and GTPV ORFs

ORF no.[a]	Accession no.[b]	SPPV TU Position	SPPV TU Length[c]	SPPV TU Changes[d] vs. SA	SPPV TU Changes[d] vs. NK	SPPV SA Length	SPPV SA Changes vs. NK	SPPV SA % Id[e] vs. PL	SPPV NK Length	GTPV PL Position	GTPV PL Length	GTPV PL Changes vs. GV	GTPV GV Length	LSDV Length	LSDV % Id vs. SA	LSDV % Id vs. PL	Predicted structure and/or function[f]	MYXV ORF[g]	VACV ORF[h]
001	M28823	589-113		Q/H			H/Q	97		648-172				159	96	99		M003.1	B15R
002	M28823													131				M003.2	
003	M28823	2167-1235	311	+72 aa	+72 aa			97		2056-1337				240	97	99	ER-localized apoptosis regulator	M004	B9R
004														57				M004.1	
005		2243-2746	168			168		92	168	2333-2842				170	93	93	IL-10		
006		3455-2763						95		3551-2859				231	96	96	IL-1R		B16R
007		4545-3481						96		4640-3576				355	98	98			C10L
008		5454-4630		I/N	I/N			94		5530-4706				275	94	95	Interferon-γ receptor	M007	B8R
009														230			α-Amanitin sensitive protein	M139R	N2L
010		6646-6161		V/A				93		6631-6146				162	95	94	LAP/PHD-finger protein	M153R	
011	S78201	7818-6697	374	A/T	A/T	374		92	374	7826-6684				381	92	95	G protein-coupled chemokine receptor		
012	S78201	8557-7925						95		8562-7930				211	95	95	Ankyrin repeat protein	M149R	B4R
013	S78201													341			IL-1R		B16R
014		9918-9652						93		9903-9637				89	96	96	eIF2α-like PKR inhibitor	M156R	K3L
015		10390-9908						94		10375-9893				161	95	96	IL-18 binding protein		
016		10698-10399	100			100		92	100	10683-10396	96		96	89	96	97	EGF-like growth factor	M010L	C11R
017		11219-10692		D/N	D/N			95		11204-10677				176	94	96	Antiapoptotic virulence factor	M011L	
018		11701-11264						99		11683-11246				146	98	97	dUTPase	M012L	F2L
019	(a)	13452-11746						93		13414-11729	562		282	569	95	95	Kelch-like protein	M014L	F3L
	(b)												231						
020		14488-13526						97		14444-13482				321	98	98	Ribonucleotide reductase, small subunit	M015L	F4L
021		14767-14531	79	A/S		79	S/A	86	79	14719-14483	79		79	86	81	82		M016L	
022		15148-14840	103			103		85	103	15114-14791	108		108	112	83	87			
023		15557-15342						95		15534-15319				72	97	97		M018L	F8L
024		16286-15639						97		16265-15618				216	98	98		M019L	F9L
025		17607-16267						99		17586-16246				447	98	99	Ser/Thr protein kinase; virus assembly	M020L	F10L
026														302					F11L
027		20390-18474	639	R/K,-N	R/K,-N	640		96	640	20429-18513	639		639	638	97	97	EEV maturation	M021L	F12L
028	AF119594	21509-20400		T/A	G/C,T/A		G/C	98		21548-20439				370	98	99	Palmitylated EEV envelope protein	M022L	F13L
029		22148-21714						99		22187-21753				145	99	100		M024L	F15L
030		22881-22225						96		22919-22263				219	97	97		M025L	F16L
031		22953-23264						95		22994-23305				104	96	95	DNA-binding virion core protein	M026L	F17L
032		24695-23274		K/N	K/N			97		24736-23315				474	97	98	Poly(A) polymerase large subunit PAP_L	M027L	E1L
033		26899-24695						97		26940-24736				735	97	97		M028L	E2L
034		27444-26914		I/M				97		27530-26955	192		192	177	97	97	dsRNA-binding PKR inhibitor	M029L	E3L
035		28172-29302	377			377		97	377	28233-29363	377		377	402	96	96		M031R	E5R
036		28087-27506	194	D/E,K/N	D/E,K/N	194		96	194	28150-27548				201	95	97	RNA polymerase subunit RPO30	M030L	E4L
037		29315-31012			S/R		S/R	98		29376-31073				566	99	98		M032R	E6R
038		31025-31819	265			265		99	265	31086-31880	265	N/D	265	266	99	99		M033R	E8R
039		34851-31822		S/L,A/S	S/L,A/S			97		34912-31883				1010	98	98	DNA polymerase	M034L	E9L
040		34885-35169			S/T		S/T	95		34946-35230				95	98	96	IMV redox protein, virus assembly	M035R	E10R
041		35561-35172						92		35622-35233				130	93	97	Virion core protein		E11L
042		37602-35551		H/N	H/N			96		37663-35612				684	97	97		M036L	O1L
043		38652-37711						99		38713-37772				314	99	99	DNA-binding virion core protein	M038L	I1L
044		38874-38662	71			71		97	71	38935-38723	71		71	72	95	95		M039L	I2L
045		39705-38878		V/I	V/I			95		39766-38939				276	96	97	DNA-binding phosphoprotein	M040L	I3L
046		40001-39768						97		40049-39816				78	100	97	IMV membrane protein	M041L	I5L
047		41203-40022						98		41251-40070				394	98	99		M042L	I6L
048		42497-41199						99		42545-41247				433	99	100	Virion core protein	M043L	I7L
049		42503-44530		M/I,D/Y	M/I,D/Y			98		42551-44578				676	97	98	NPH-II, RNA helicase	M044R	I8R
050		46317-44533	595			595		98	595	46365-44581	595		595	596	98	98	Metalloprotease, virion morphogenesis	M045L	G1L
051		46640-47305						98		46688-47353				222	98	100	Putative transcriptional elongation factor	M047R	G2R
052		46646-46317						99		46694-46365				110	95	96		M046L	G3L
053		47652-47275						98		47700-47323				126	99	99	Glutaredoxin 2, virion morphogenesis	M048L	G4L

054		47655-48965						97		47703-49013				437	99	97		M049R	G5R
055		48980-49168						100		49017-49205				63	100	100	RNA polymerase subunit RPO7	M050R	G5.5R
056		49171-49692						97		49208-49729				174	97	97		M051R	G6R
057		50830-49712		N/T	N/T			96		50867-49749				373	97	98	Virion core protein	M052L	G7L
058		50861-51640		K/Q			K/Q	99		50898-51677				260	99	100	Late transcription factor VLTF-1	M053R	G8R
059		51670-52680	337	I/L	I/L,W/R	337	W/R	94	337	51707-52714				336	95	95	Myristylated protein	M054R	G9R
060		52684-53418						97		52718-53452				245	98	99	Myristylated IMV envelope protein	M055R	L1R
061		53459-53734						95		53484-53759				92	92	96		M056R	L2R
062		54710-53757						97		54728-53775				318	99	98		M057L	L3L
063		54735-55493						97		54753-55511				253	99	98	DNA-binding virion core protein VP8	M058R	L4R
064	D00423	55512-55907	132			132		96	132	55530-55922				131	98	97	Putative membrane protein	M059R	L5R
065	D00423	55864-56304						98		55882-56322				147	98	98		M060R	J1R
066	D00423	56332-56862						96		56353-56883				177	98	97	Thymidine kinase	M061R	J2R
067	D00423	56938-57528	197			197		96	197	56959-57546	196	N/D	196	198	96	96	Host range protein	M062R	C7L
068	D00423	57592-58590						98		57608-58606				333	99	99	Poly(A) polymerase small subunit PAP_S	M065R	J3R
069		58508-59062						98		58524-59078				185	98	99	RNA polymerase subunit RPO22	M066R	J4R
070		59472-59074						99		59488-59090				133	100	99		M067L	J5L
071	AF124517	59559-63413			T/A		T/A	99		59574-63428				1285	99	98	RNA polymerase subunit RPO147	M068R	J6R
072	AF124517	63936-63424						98		63950-63438				171	98	98	Protein phosphatase, virus assembly	M069L	H1L
073	AF124517	63952-64521						99		63966-64535				190	100	99		M070R	H2R
074	AF124517	65492-64524	323			323		97	323	65503-64538				322	98	97	IMV envelope protein p35	M071L	H3L
075	AF124517	67919-65526						98		67929-65536				798	98	98	RNA polymerase-associated, RAP94	M072L	H4L
076		68063-68746	228	+ND	V/I,+2N	226	V/I	85	226	68071-68790	240		240	223	95	86	Late transcription factor VLTF-4	M073R	H5R
077		68791-69741						99		68835-69785				317	99	99	DNA topoisomerase	M074R	H6R
078		69764-70204						98		69808-70248				147	98	100		M075R	H7R
079		70239-72764						98		70279-72804				842	98	98	mRNA capping enzyme, large subunit	M076R	D1R
080		73196-72732						96		73236-72772				155	97	98	Virion protein	M077L	D2L
081		73198-73932		N/H,S/A	N/H,S/A			97		73238-73972				245	97	98	Virion protein	M078R	D3R
082		73932-74585						98		73972-74625				218	97	98	Uracil DNA glycosylase	M079R	D4R
083		74631-76988						99		74671-77028				786	98	98	NTPase; DNA replication	M080R	D5R
084		76988-78892		D/E	D/E			98		77028-78932				635	99	99	Early transcription factor $VETF_S$	M081R	D6R
085		78920-79408						100		78960-79448				163	99	99	RNA polymerase subunit RPO18	M082R	D7R
086		79414-80091	226	N/K	N/K	226		96	226	79492-80130				213	96	97	*mutT* motif	M084R	D9R
087		80094-80852						96		80133-80891				253	98	96	*mutT* motif; gene expression regulator	M085R	D10R
088		82769-80865						98		82807-80903				635	98	99	NPH-I; transcription termination factor	M086L	D11L
089		83659-82799		E/K	E/K			98		83695-82835				287	98	99	mRNA capping enzyme, small subunit	M087L	D12L
090		85349-83703						99		85384-83738				549	99	99	Rifampin resistance, IMV assembly	M088L	D13L
091		85828-85379		V/F	V/F			96		85863-85414				150	96	96	Late transcription factor VLTF-2	M089L	A1L
092		86556-85861						99		86591-85896				232	100	99	Late transcription factor VLTF-3	M090L	A2L
093		86780-86556						98		86815-86591				75	98	100		M091L	8.9kd
094		88774-86792						99		88806-86827	660		660	661	99	99	Virion core protein P4b	M092L	A3L
095		89335-88853						93		89367-88885				161	97	94	Virion core protein, virion assembly	M093L	A4L
096		89376-89882	169	+E	+NK	168	+E	97	167	89408-89908	167		167	170	97	97	RNA polymerase subunit RPO19	M094R	A5R
097		91009-89885		S/N	S/N			97		91035-89911				375	97	98		M095L	A6L
098		93174-91033						98		93200-91059				714	98	98	Early transcription factor $VETF_L$	M096L	A7L
099		93230-94099						98		93257-94126				290	98	98	Intermediate transcription factor VITF-3	M097R	A8R
100		94354-94121						97		94374-94141				78	98	98	IMV membrane protein	M098L	A9L
101		97069-94358			M/T,S/T		M/T,S/T	97		97089-94378		M/T		904	98	98	Virion core protein P4a	M099L	A10L
102		97084-98034						96		97104-98054				317	97	99		M100R	A11R
103		98606-98037						97		98626-98057				190	97	97	Virion core protein	M101L	A12L
104		98862-98662						91		98889-98689				67	94	97	IMV membrane protein	M102L	A13L
105		99233-98949						100		99258-98974				95	100	100	IMV membrane protein	M103L	A14L
106		99411-99253						100		99436-99278				53	100	100	IMV membrane virulence factor	M104L	A14.5
107		99688-99404						96		99713-99429				95	100	96		M105L	A15L
108		100805-99675						97		100830-99700				377	98	98	Myristylated protein	M106L	A16L
109		101405-100824	194			194		94	194	101430-100849	194		194	196	93	94	Phosphorylated IMV membrane protein	M107L	A17L
110		101420-102859		I/M	I/M			96		101445-102884				480	97	97	DNA helicase; transcriptional elongation	M108R	A18R
111		103067-102846						97		103092-102871				74	97	100		M109L	A19L
112		103414-104703			V/I		V/I	96		103439-104728				430	98	98	DNA polymerase processivity factor	M111R	A20R
113		103415-103071						97		103440-103096				115	98	99		M110L	A21L
114		104675-105178						97		104700-105236	179		179	168	98	98	Holliday junction resolvase	M112R	A22R
115		105206-106360		D/Y	D/Y			98		105232-106386				385	98	98	Intermediate transcription factor VITF-3	M113R	A23R

TABLE 1 - *Continued*

ORF no.[a]	Accession no.[b]	SPPV TU Position	SPPV TU Length[c]	SPPV TU Changes[d] vs. SA	SPPV TU Changes[d] vs. NK	SPPV SA Length	SPPV SA Changes vs. NK	SPPV SA % Id[e] vs. PL	SPPV NK Length	GTPV PL Position	GTPV PL Length	GTPV PL Changes vs. GV	GTPV GV Length	LSDV Length	LSDV % Id vs. SA	LSDV % Id vs. PL	Predicted structure and/or function[f]	MYXV ORF[g]	VACV ORF[h]
116	M30039	106394-109861		I/V			V/I	99		106420-109887				1156	99	99	RNA polymerase subunit RPO132	M114R	A24R
117	M30039	110452-110006	149			149		93	149	110350-109907				148	92	95	Fusion protein, virus assembly	M115L	A27L
118	M30039	110875-110456						98		110773-110354				140	100	98		M116L	A28L
119	M30039	111787-110879	303			303		99	303	111685-110777	303		303	302	98	98	RNA polymerase subunit RPO35	M117L	A29L
120		111980-111759						98		111878-111657				74	100	98	IMV membrane	M118L	A30L
121		112925-112158	256			256		99	256	112822-112061				254	99	98	DNA packaging, virus assembly	M120L	A32L
122		113059-113643	195	-E	-E			93		112956-113546	197		197	196	95	95	EEV glycoprotein	M121R	A33R
123		113679-114191						95		113579-114091				171	97	92	EEV glycoprotein	M122R	A34R
124		114222-114794						95		114123-114695				191	95	96		M123R	A35R
125		114834-115697						98		114735-115598				288	99	98		M124R	
126		115756-116271	172	S/P	S/P	172		93	172	115661-116176	172		172	181	91	91	EEV glycoprotein	M125R	A36R
127		116285-117103						97		116190-117008				273	98	97		M126R	A37R
128		118015-117113	301			301		93	301	117917-117018				300	95	97	CD47-like protein	M128L	A38L
129		118114-118431	106			106		82	106	118014-118361	116		116	123	83	90		M130R	
130		118503-118733	77			77		92	77	118434-118676				81	94	95			
131		118802-119284		I/V	I/V			97		118735-119217				161	98	99	Superoxide dismutase-like protein	M131R	A45R
132														176					
133		119900-121570	557	V/I	V/I	557		95	557	119776-121452				559	96	97	DNA ligase	M133R	A50R
134		121690-127767	2026	N/D,S/A,A/S	N/D,S/A,A/S	2026		95	2026	121575-127595	2007		2007	2025	96	97	VARV B22R homologue	M134R	
135		127833-128912						90		127668-128747				360	93	91	Interferon-α/β binding protein	M135R	B19R
136	(a)									128794-129063	90		90	153		87		M136R	K7R
137		129452-130456						97		129313-130317				335	97	97		M137R	A51R
138		130493-131032	180			180		90	180	130350-130907				186	78	80	Ig domain, OX-2-like protein	M141R	
139		131074-131988						96		130949-131863				305	97	98	Ser/Thr protein kinase, DNA replication	M142R	B1R
140		132022-132741						94		131896-132615				240	94	95	RING finger protein, host range	M143R	
141		132794-133471	226			226		94	226	132667-133341				225	95	97	EEV host range protein	M144R	B5R
142		133476-133880	135			135		88	135	133346-133750	135		135	134	90	91	Secreted virulence factor	M146R	N1L
143		133920-134825						95		133790-134695				302	97	97	Tyrosine protein kinase-like protein	M147R	
144		134992-136632		T/S	T/S, D/N		D/N	95		134820-136460		+FL	545	547	95	95	Kelch-like protein	M140R	A55R
145	(a)	136663-138576	638			638		96	250	136490-138412	641		641	634	96	97	Ankyrin repeat protein	M148R	B4R
	(b)								371										
146		138700-139929	410			410		96	410	138538-139773	412		412	413	96	95	Phospholipase D-like protein	M022L	K4L
147		139997-141490		I/L,K/T	I/L,K/T			95		139831-141324				498	96	96	Ankyrin repeat protein	M149R	B4R
148	(a)	141541-142881		E/K				93	116	141374-142714				447	94	95	Ankyrin repeat protein	M148R	B4R
	(b)								178										
149		142904-143914		L/I	L/I			94		142739-143749				337	94	95	Serpin	M151R	C12L
150		143956-144438						94		143791-144273				161	93	99		M139R	A52R
151		144482-146137	552			552		94	552	144317-145972	552	N/H	552	550	95	95	Kelch-like protein	M140R	A55R
152		146213-147676	488	N/D	N/D	484		92	484	146045-147403	453		453	489	95	95	Ankyrin repeat protein	M005	B4R
153														91				M004.1	
154	M28823	147789-148721	311	+72 aa	+72 aa			97		147880-148599				240	97	99	ER-localized apoptosis regulator	M004	B9R
155	M28823													131				M003.2	
156	M28823	149367-149843		Q/H			H/Q	97		149288-149764				159	96	99		M003.1	B15R

[a] CaPV ORF number. Boldface numbers indicate ORFs disrupted in SGPV but present in LSDV (accession no. AF325528). (a) and (b) indicate ORF fragments in specific viruses.

[b] GenBank database accession numbers of homologous SGPV sequences.

[c] Length of ORF in codons. SGPV ORF lengths were presented only if different from lengths of LSDV homologues. Boxes indicate CaPV ORFs fragmented in vaccine viruses.

[d] Changes, amino acid substitutions (/), insertions (+), and deletions (-) in single letter code, aa, amino acids.

[e] % Id, Percent amino acid identity.

[f] Function was deduced from the degree of similarity to known genes and from Prosite signatures. ER, endoplasmic reticulum; EEV, extracellular enveloped virion; IMV, intracellular mature virion; VARV, variola virus.

[g] Best matching ORF from the MYXV genome (accession no. AF170726).

[h] Best matching ORF from the VACV strain Copenhagen genome (accession no. M35027), with the exception of the VACV strain Western Reserve 8.9-kda protein (accession no. P07608) (ORF 093) frameshifted in VACV strain Copenhagen.

TABLE 2. Comparisons between CaPV genomes[a]

Genome	Identity				
	SPPV			GTPV	
	TU	SA	NK	PL	GV
SA	**193-131 (99.7)**				
NK	**188-137 (99.8)**	**71-36 (99.9)**			
PL	2939-1972 (96.2)	2949-1979 (96.2)	2960-1986 (96.2)		
GV	2945-1974 (96.2)	2955-1980 (96.2)	2964-1987 (96.2)	**7-4 (100.0)**	
LSDV	2603-1816 (96.8)	2617-1822 (96.9)	2615-1825 (97.0)	2373-1676 (97.2)	2380-1676 (97.2)

[a] Pairwise comparisons of CaPV genomes presented as total differences–single-nucleotide substitutions (percent nucleotide identity rounded to the nearest 0.1%). Boldface numbers reflect intraspecies comparisons. Changes were calculated with the Diffseq program (ftp:uk.embnet.org/pub/EMBOSS).

upstream insertions resulting in frameshifts. Additionally, GTPV contains a 90-amino-acid ORF (ORF 136a) that is homologous to the carboxyl terminus of LSDV136. This ORF is absent in SPPV.

Differences between SPPV and GTPV in amino acid identity and ORF size are greater in terminal genomic regions and in genes with likely virulence and host range functions. Seventy-six percent of the most variable ORFs (<96% amino acid identity) and 54% of the ORFs differing in size between SPPV and GTPV occur in the terminal 35% of the genome (ORFs 001 to 023 and ORFs 122 to 156) (Table 1). Of the seven least similar ORFs (82 to 90% amino acid identity), three are similar to myxoma virus (MYXV) M016L (ORF 021), vaccinia virus (VACV) interferon-α/β binding protein (ORF 135), and VACV N1L-secreted virulence factor (ORF 142), and four differ in size (4 to 14 amino acids) and include a CaPV-specific ORF (ORF 022) and homologues of late transcription factor 4 (ORF 076), MYXV M130R (ORF 129) and an OX-2-like immunoglobulin (Ig) domain protein (ORF 138) (Table 1). Size differences (4 to 19 amino acids) are also present between SPPV and GTPV homologues of CC chemokine receptor (ORF 011), epidermal growth factor (EGF)-like growth factor (ORF 016), kelch-like protein (ORF 019), double-stranded RNA (dsRNA)-binding PKR inhibitor (ORF 035), RPO30 (ORF 036), VACV D9R *mutT* motif protein (ORF 086), Holliday junction resolvase (ORF 114), variola virus B22R (ORF 134), and ankyrin repeat protein (ORF 152). These differences in proteins located in terminal genomic regions likely affect aspects of viral virulence and host range.

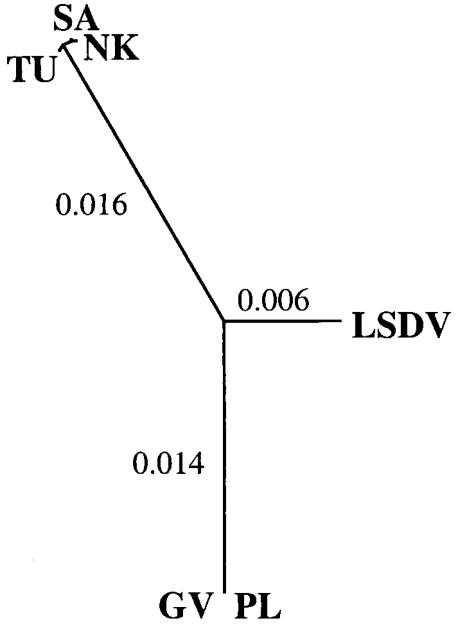

FIG. 1. Phylogenetic comparison of CaPVs. Genomic nucleotide sequences excluding terminal repetition were aligned by using Dialign (42) to generate the unrooted tree. The maximum-likelihood algorithm with HKY correction for multiple substitutions was used as implemented by the Phylip package (22). Branch length values indicate changes per nucleotide. Similar results were obtained by using the maximum-parsimony algorithm and the neighbor-joining algorithm, which maintained 100% support for species-specific groupings after 1,000 bootstrap replicates (data not shown).

Comparison of SGPV and LSDV. Nine LSDV genes with likely virulence and host range functions are disrupted in SPPV and GTPV (Table 1). Genes affected by insertions, deletions, and substitutions include an LSDV-specific gene (LSDV132) and those similar to IL-1 receptor (IL-1R) (LSDV013), MYXV M003.2 (LSDV002 and LSDV155), MYXV M004.1 (LSDV004 and LSDV153), VACV N2L (LSDV009), VACV F11L (LSDV026), and VACV K7L (LSDV136) genes (24) (Table 1). Affected SGPV genes are highly fragmented, with 4 to 23 potentially frameshifting nucleotide changes per ORF compared to LSDV. The number of genomic changes relative to LSDV in these nine SGPV genomic regions is relatively high (average of 2.6 nucleotide insertion/deletion sites per 100 bases) compared to changes throughout the remainder of the genome (average of 0.09 nucleotide insertion/deletion sites per 100 bases). Although ORF 136 is highly fragmented in SGPV, two-thirds of the predicted protein remains as a carboxyl-terminal fragment in GTPV (Table 1). Given the likely ancestral nature of several LSDV genes disrupted in SGPV (present in other poxvirus genera), available data suggest that gene fragmentation and sequence divergence occurred during adaptation of an LSDV-like ancestor to sheep and/or goats.

Extensive fragmentation of these nine ORFs in SGPV likely results in functional inactivation. Of the nine genes disrupted, only the IL-1R gene (ORF 013) is orthologous to genes of known function, including VACV WR B15R homologues, secreted proteins that bind and inactivate host IL-1β to affect viral virulence (6, 56). LSDV013 contains the three Ig domains common to IL-1R and likely functions as an IL-1 binding protein (59). Disruption of ORF 013 in SGPV likely affects the ability of the virus to modulate host IL-1-mediated responses.

ORF 009 is similar to VACV N2L, an ORF associated with viral sensitivity to α-amanitin (57). ORF 136 has limited similarity to a previously described poxvirus gene family, which includes VACV A52R, an antagonist for host cell IL-1 and Toll-like receptor-mediated intracellular signaling (9, 55, 59). The absence of these genes in SGPV likely affects aspects of CaPV virulence and host range and suggests a specific role for them in bovine host range.

ORFs 002 and 155 and ORF 013, disrupted in all SGPVs described here and in an Indian strain of SPPV, are intact in both the Kenyan "sheep and goat poxvirus" 0240 strain (KS-1) and LSDV (Table 1) (11, 16, 17, 24). Strain 0240 yields restriction patterns very similar to LSDV, and it causes mild disease in sheep and goats (8, 32, 33). Notably, published sequences from strain 0240 are more similar to LSDV (99.6 to 99.8% nucleotide identity) than to SPPV (92.0 to 97.1% nucleotide identity). Although considered of low virulence in cattle, strain 0240 induced more severe reactions than other SPPV and GTPV strains when administered experimentally, and it caused LSDV-like disease when used as a vaccine (8, 32, 60). These data suggest that strain 0240 may in fact be LSDV (32).

Comparison of SGPV field and vaccine strains. SPPV NK and GTPV GV vaccine strains have greater than 99.9% amino acid identity to their respective field strains (Table 2). SPPV NK contains only 71 genomic differences compared to SPPV SA, including 36 single-nucleotide substitutions, 15 insertions of 1 to 29 nucleotides, and 20 deletions of 1 to 4 nucleotides and affecting 17 proteins (Table 1). GTPV GV is extremely similar to the PL field isolate; differences occur in only seven genomic locations and include four single-nucleotide substitutions, two single-nucleotide insertions, and one deletion of 28 nucleotides and affect six proteins. Comparative genomic data suggest that these genomic changes account for viral attenuation and that the changes affect genes with predicted virulence and host range functions (Table 1).

In SPPV NK, single in-frame stop and frameshift mutations are present in ankyrin repeat-containing genes ORF 145 and ORF 148, respectively. Each gene is represented by two smaller ORFs in NK (ORFs 145a and 145b and ORFs 148a and 148b). Poxvirus ankyrin repeat genes have been associated with host range functions in orthopoxviruses and leporipoxviruses, and they may inhibit virally induced apoptosis (31, 44, 48). It has also been suggested that specific complements of ankyrin repeat genes may affect poxvirus host range (7, 54). The attenuated phenotype of the VACV Ankara strain may be due in part to mutations in ankyrin repeat genes (7). Ankyrin repeat motifs in other proteins are clearly involved in mediating protein-protein interactions, and the VACV K1L ankyrin repeat host range protein interacts with at least one other viral protein (41, 52). Disruption of two of the five ankyrin repeat genes in SPPV vaccine strain NK further suggests a significant role for them in viral virulence and/or host range.

GTPV vaccine strain GV contains mutations in all genes encoding kelch-like family proteins (ORFs 019, 144, and 151). ORF 019 contains a single frameshift that results in two smaller ORFs (ORFs 019a and 019b), ORF 144 contains a 28-nucleotide deletion in the carboxyl and 3′-noncoding region, and ORF 151 contains a single nonconservative amino acid substitution (N to H). These three ORFs in CaPV field isolates contain four to five imperfect carboxyl-terminal repeats similar to those found in the *Drosophila* kelch protein and to kelch-like proteins present in several ChPV genera (4, 10, 40, 53). Kelch and poxviral kelch-like proteins also contain an amino-terminal domain (BTB/POZ domain) known to mediate oligomerization and protein complex formation in numerous cellular actin-binding and transcriptional regulatory proteins (15, 53). Kelch repeat motifs are found in functionally diverse proteins known to affect cellular transcription, development, and organization, and they are involved in protein-protein interactions (1). Notably, proteins from influenza virus and herpes simplex virus bind cellular kelch repeat proteins and affect viral pathogenesis (1). The function of poxvirus kelch-like proteins is unknown; however, they are nonessential for replication of VACV in cell culture and have been speculated to mediate viral interaction with specific cellular components (39, 49, 54). The changes observed in all kelch-like proteins of the GTPV vaccine strain GV may be highly significant for viral attenuation. Overall, data from attenuated CaPVs suggest that further elucidation of functions of ankyrin repeat and kelch-like proteins may be helpful in design of more effective and versatile CaPV vaccines.

Conclusions. Genome sequences of SPPV and GTPV described here, together with their comparison to the complete genomic sequence of LSDV, provides a comprehensive view of CaPV genomics. CaPV genomes are very similar to each other, averaging no less than 96% nucleotide identity over their entire length. SPPV, GTPV, and LSDV contain the same repertoire of orthologous genes, with the exception that SPPV and GTPV lack nine LSDV genes with likely CaPV virulence and host range functions. SPPV and GTPV genomes sequenced here are phylogenetically distinct from each other and from LSDV, and they contain species-specific nucleotide differences that may be associated with aspects of host range. Relatively few genomic changes in SPPV and GTPV vaccine viruses account for viral attenuation.

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