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Title: Extended sequencing of vaccine and wild-type capripoxvirus isolates provides insights into genes modulating virulence and host range

Running Title: Capripoxvirus virulence and host range

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SUMMARY

The genus *Capripoxvirus* in the subfamily *Chordopoxvirinae*, family *Poxviridae*, comprises sheeppox virus (SPPV), goatpox virus (GTPV), and lumpy skin disease virus (LSDV), which cause the eponymous diseases across parts of Africa, the Middle East, and Asia. These diseases cause significant economic losses and can have a devastating impact on the livelihoods and food security of small farm holders. So far, only live classically attenuated SPPV, GTPV, and LSDV vaccines are commercially available and the history, safety, and efficacy of many have not been well established. Here we report 13 new capripoxvirus genome sequences, including the hairpin telomeres, from both pathogenic field isolates and vaccine strains. We have also updated the genome annotations to incorporate recent advances in our understanding of poxvirus biology. These new genomes and genes grouped phenetically with other previously sequenced capripoxvirus strains and these new alignments collectively identified several recurring alterations in genes thought to modulate virulence and host range. In particular, some of the many large capripoxvirus ankyrin and kelch-like proteins are commonly mutated in vaccine strains, while the variola virus B22R-like gene homolog has also been disrupted in many vaccine isolates. Among these vaccine isolates, frameshift mutations are especially common and clearly present a risk of reversion to wildtype in vaccines bearing these mutations. A consistent pattern of gene inactivation from LSDV to GTPV and then SPPV is also observed, much like the pattern of gene loss in orthopoxviruses but, rather surprisingly, the overall genome size of ~150 kbp

remains relatively constant. These data provide new insights into the evolution of capripoxviruses and the determinants of pathogenicity and host range. They will find application in the development of new vaccines with better safety, efficacy, and trade profiles.

Key Words:

Poxvirus, goatpox virus, sheeppox virus, lumpy skin disease virus, capripoxvirus, virulence

INTRODUCTION

The genus *Capripoxvirus*, subfamily *Chordopoxvirinae*, family *Poxviridae*, comprises sheeppox virus (SPPV), goatpox virus (GTPV), and lumpy skin disease virus (LSDV). These cause disease in sheep, goats, and cattle, respectively, and LSDV can also infect some wild African game species (Young, Basson, & Weiss, 1970). LSDV is endemic throughout Africa except for some north African countries (Morocco, Algeria, Tunisia and Libya). Recent outbreaks of lumpy skin disease have also been observed throughout the Middle East, with spread to Eastern European countries (Greece, Bulgaria, Serbia, Montenegro, the former Yugoslav Republic of Macedonia, Kosovo and Albania), and in parts of Asia (Azerbaijan, Armenia, Georgia, Kazakhstan, and Russia) (Tuppurainen, Babiuk, & Klement, 2018; Tuppurainen et al., 2017). GTPV and SPPV are widespread, found across north and central Africa, the Middle East, the Indian subcontinent, and in parts of Asia including China, Russia, and Vietnam (Tuppurainen et al., 2017). Recent outbreaks of sheeppox and goatpox, as well as lumpy skin disease, have also been reported in Greece, the first incursion of these viruses in European Union territory (Tuppurainen et al., 2017). SPPV and GTPV are generally host specific, although a few have been shown to infect and cause disease in both sheep and goats (Kitching & Taylor, 1985a); LSDV primarily infects cattle. All of these viruses are closely related to each other (across the conserved core LSDV is >98% identical to GTPV and SPPV, while SPPV is >97% identical to GTPV) and can only be confidently differentiated by molecular characterization (Tulman et al., 2002).

These diseases can cause significant economic losses (Babiuk, Bowden, Boyle, Wallace, & Kitching, 2008). Permanent scars reduce the value of hides and both milk and meat production are reduced (Yeruham et al., 2007). Efforts to control the spread of disease also create barriers to trade (Babiuk et al., 2008; Tuppurainen et al., 2017). Morbidity rates in LSDV infections vary greatly (5-45%) and up to 10% mortality has been observed (Babiuk et al., 2008). GTPV and

SPPV infections cause more serious disease with 70-90% morbidity in adult animals and mortality rates approaching 50% in adults and up to 100% in the young (Bhanuprakash, Indrani, Hosamani, & Singh, 2006). An interesting feature of these diseases is that virulence varies with the breed and species of animal and virus isolate. For goatpox and sheeppox, disease is generally milder in healthy adult indigenous breeds and more severe in European goat and sheep breeds; this has hampered efforts at advancing agricultural production (Babiuk et al., 2008; Bhanuprakash et al., 2006; Tuppurainen et al., 2017). Some effective live-attenuated vaccines against capripoxvirus infections are in use (Roth & Spickler, 2003); particular vaccines provide immunity for up to two years and are effective in endemic areas (Kitching, 2003). However, few of the live-attenuated vaccines are supported by standardized challenge trial data [exceptions include (Ben-Gera, Klement, Khinich, Stram, & Shpigel, 2015; Gari et al., 2015)]. Not surprisingly, vaccine failures and vaccine-induced disease have been observed (Hunter & Wallace, 2001). Moreover, confusion caused by the biological origins of some vaccines has also been reported (Tuppurainen et al., 2014). There is a need for comprehensive databases of sequences to aid in the design of simple and effective molecular tools that can be used to classify capripoxviruses and to track vaccine strains in production and in the field. Genomics-guided genetic engineering can also offer another way of producing better defined, more stable, and more effective capripoxvirus vaccines.

Other approaches to vaccination may be possible including transgenic viruses, which also offer a route for producing heterologous protection against diseases such as rabies (Aspden et al., 2002), peste des petits ruminants (Berhe et al., 2003; Fakri et al., 2018), and Rift Valley fever (Wallace et al., 2006). The use of a specifically modified capripoxvirus as a live attenuated vaccine has been proposed and demonstrated as feasible by inactivating the kelch-like gene (SPPV_019) in SPPV-SA (Balinsky et al., 2007). More recently Zhang *et al.* showed that deleting a putative NF- κ B inhibitor (GTPV_135) enhanced antibody responses to a GTPV-encoded peste des petits ruminants hemagglutinin (Zhang, Sun, Chen, & Bu, 2018). A long-term goal in such vaccine development programs would be to gain a better understanding of factors affecting the host range of these viruses. Antigenic similarity across capripoxviruses and the ability to express heterologous antigen highlight a potential for the development of genus-wide vaccine strains that could be used to protect against a broad range of diseases (Kitching, 2003).

These investigations depend upon an understanding of capripoxvirus genome sequences. The complete genomes of LSDV (Tulman et al., 2001) and GTPV and SPPV (Tulman et al., 2002) were first reported nearly 20 years ago. Since then additional LSDV genome sequences have been reported, but the databases are less well populated with examples of SPPV and GTPV. We have therefore determined the sequence of new SPPV and GTPV strains collected from different parts of the world. By comparing these and previously reported capripoxvirus genome sequences our study offers additional insights into factors affecting both virulence and the gene loss that probably underlies the evolution/speciation and altered host range of capripoxviruses.

MATERIALS AND METHODS

Virus strains. Table 1 summarizes the virus isolates sequenced for this report. Kitching and Taylor (Kitching & Taylor, 1985a, 1985b)(Kitching, 1985) describe the year of isolation and the source of many of these viruses. The Vietnamese GTPV was acquired by the National Centre for Foreign Animal Disease in 2006 and the remaining viruses came from the collection of the Institute for Animal Health, Pirbright Laboratory. Further characterization using experimental infection has been undertaken for NIG-SPPV and IN-GTPV (Bowden, Babiuk, Parkyn, Copps, & Boyle, 2008), and YE-GTPV and VI-GTPV (Babiuk, Bowden, et al., 2009). The OM-GTPV was isolated from the Sultanate of Oman in 1984 (Kitching & Mellor, 1986). The origins of the Pendik, SAU vaccine, and AG isolates of SPPV are less certain; but all of them are probably vaccine isolates (Black, Hammond, & Kitching, 1986; Kitching, 1986).

Virus culture, DNA isolation and sequencing. All of the viruses were cultured on *Ovis aries* sheep testes (OA3.T) cells in BSL3Ag containment at the National Centre for Foreign Animal Disease-Canadian Food Inspection Agency, Winnipeg, Canada (CFIA-NCFAD). The long-term passage history is not known with certainty. Most stocks are reported to have been passaged 1-3 times when first isolated and were subsequently passaged 2-3 times to prepare DNA for sequencing. None were plaque purified to minimize the risk of selecting a mutant arising through passage in vitro. The viruses were harvested by freeze-thawing the cells three times and purified and concentrated using ultracentrifugation as previously described (Babiuk, Wallace, et al., 2009). The DNA was extracted using phenol chloroform (Black et al., 1986) and then processed

using either an Illumina Neoprep system with TruSeq chemistry, or a Nextera XT “Tagmentation” kit. Illumina GAIIx or MiSeq platforms were used to sequence the products. CLC Genomics (v. 11) software was used to assemble the reads into full-length genomes using both *de novo* and “map-to-reference” methods depending upon the read depth. In the latter case if any mutations were detected, compared to the reference, the experimentally-determined consensus was adopted. In *de novo* sequencing at least one of the inverted terminal repeats generally assembled as a separate contig and was later joined to produce the full-length genome sequence. Sequences encoding the hairpin ends were retrieved using the method described by Noyce et al. (Noyce, Lederman, & Evans, 2018) and were appended manually. The sequences have been submitted to GenBank (Table 1).

Genome annotation. The “Genome Annotation and Transfer Utility” [GATU, (Tcherepanov, Ehlers, & Upton, 2006)] was used to transpose the gene annotations from vaccinia virus strain Western Reserve (AY243312) to LSDV isolate KE and then SnapGene (v. 4.2) was used to manually update the gene annotations using homology searches on the NCBI “Basic Local Alignments Search Tool” (BLAST) server. This updated annotation of LSDV strain KE was used as the template to produce consistent annotation of the capripoxvirus genome sequences reported here, again using GATU (<https://4virology.net/virology-ca-tools/gatu/>). ORFs of less than fifty amino acids in length were omitted, unless previously characterised or detected in multiple species.

Genome and other gene comparisons. Phenetic comparisons were performed on 36 complete genomes using the k-mer method (Deraspe et al., 2017) and a software package included in CLC genomics. Table 2 lists the sequences retrieved from GenBank. For the k-mer method, default settings were used except k-mer of 8 (optimised for the small differences between genome sequences being compared). Note, two SPPV genomes (KT438550 and KT438551) were reoriented (reverse and complement) to enable comparisons with the other genome sequences. The maximum likelihood phylogeny (Figure 5) was produced using UPGMA as the construction method with a bootstrap setting of 100. Tests of other CLC subroutines (e.g. using the k-mer method) produced essentially identical phenetic trees. Multiple sequence alignments were produced using Base-by-Base (Brodie, Smith, Roper, Tcherepanov, & Upton, 2004) and

MUSCLE (Edgar, 2004). Genome sequences were aligned using LAGAN (Brudno et al., 2003). M-fold (Zuker, 2003) was used to model the structures of the mismatched hairpin loops.

Ethics statement. All work with infectious sheeppox, goatpox, and lumpy skin disease viruses was performed within the CFIA-NCFAD facility located at 1015 Arlington Street, Winnipeg, Manitoba, Canada. The facility is accredited to work with these three agents through the Office of Biohazard Containment and Safety, in accordance with the Canadian Biosafety Standard, 2nd edition (2015). Other ethics approvals were not required as no original data/samples have been collected from animals or humans.

RESULTS AND DISCUSSION

New SPPV, GTPV and LSDV genomes. To broaden our understanding of capripoxvirus pathogenesis and genomics we determined the complete sequences of six GTPV and six SPPV strains, and one new LSDV genome. These strains come from Asia, India, the Middle East, or Africa and the collection included both vaccine and field strains. These viruses were cultured *in vitro* and then harvested, purified, sequenced, and genome assembly conducted as described in the Materials and Methods. The percentage of reads that mapped to a relevant reference sequence varied from 10-80% and average depth of coverage ranged from 110-to-5000 fold. In some situations, *de novo* assembly was also used to verify the sequence. In many cases we were able to extract sequences encompassing the flip and flop terminal hairpins (Noyce et al., 2018). Table 1 summarizes the viruses reported in this document. This includes six GTPV field isolates [strains from Turkey (TU), India (IN), Vietnam (VI), Yemen (YE), Sudan (SU), and Oman (OM)], as well as two SPPV field isolates [from Saudi Arabia (SAU) and Nigeria (NIG)]. We also sequenced four SPPV vaccine strains that originated in Saudi Arabia (SAU-Vac), Turkey (TU-Vac), Turkey (PE), and Iraq (AG).

Little is known concerning the concatemer resolution sites (CRS) (Merchlinsky & Moss, 1989) and the hairpin telomeres (Baroudy, Venkatesan, & Moss, 1982) of capripoxviruses, with just one report describing what appears to be a functional SPPV CRS (Gershon & Black, 1989; Merchlinsky, 1990). Where these sequences could be fully determined we “unfolded” the CRS and hairpin and attached a sequence comprising the CRS (bottom strand) + the unfolded hairpin

+ CRS (top strand) onto each end of the genome. The leftmost nucleotide was numbered as 1. Among the complete genome sequences, genome size is highly conserved; among the new SPPV and GTPV genome sequences the size is 150,000±600 bp (Table 1). As noted previously (Tulman et al., 2002), all of the new GTPV and SPPV genomes have a high (75%) A+T content.

We also assembled the sequence of a new Kenya isolate of LSDV (KE-LSDV) and its inverted terminal repeats. It also exhibited a high (74%) A+T content. In order to simplify comparisons between SPPV, GTPV and LSDV isolates, we used GATU (Tcherepanov et al., 2006) to transpose the gene annotations onto each new strain using KE-LSDV and vaccinia virus strain Western Reserve as reference genomes. The assumed gene function(s) were also updated where new discoveries permitted. Because of the close similarity between strain KE and NI-2490 (AF325528), we mostly retained the original gene numbering system proposed by Tulman *et al.* (Tulman et al., 2001). This approach creates gaps in the gene numbering in SPPV and GTPV strains, where genes are fragmented or missing.

In annotating the new capripoxvirus genomes we discovered two short open reading frames located between genes 28/29 and 120/121, which potentially encode 49 a.a. and 41 a.a. proteins, respectively. The two open reading frames were numbered genes 28.5 and 120.5. A tblastn search showed that these two encoded protein features are highly conserved at syntenic locations in LSDV, GTPV, and SPPV. Moreover, the vaccinia virus F14.5L gene encodes a 49 a.a. intracellular mature virus envelope protein (Izmailyan & Chang, 2008) which occupies the same syntenic location and shares 32% amino acid identity with the LSD|Kenya-028.5 protein. We also reassigned the more probable start codon for the LSDV homolog of vaccinia virus E5R (LSD|Kenya-036), which led to the renumbering of what had been called ORFs 035 and 036 in GenBank entry AF325528.

CRS and hairpin nucleotide sequences. Illumina-based sequencing methods can capture sequences encoding the CRS and hairpin ends of poxviruses, and these can be extracted using appropriate sequence retrieval methods (Noyce et al., 2018). The six different GTPV isolates all encoded the same putative CRS element (5'-ATTATAGGCTAAAAAAAAAGTA-3') and this differed only slightly from that encoded by six SPPV isolates (5'-ATTATAGGC-TTAAAAAAAAAGTA-3') (Figure 1A). Both sequences resemble the well-characterized CRS element in vaccinia virus (5'-ATTAGTGTCTAGAAAAAATGTG-3') (Merchinsky, 1990;

Merchlsinsky & Moss, 1989). The CRS element is essentially a late poxvirus promoter and transcription initiating at these sites likely plays a role in promoting cruciform extrusion and subsequent resolution. Unlike these highly conserved CRS sequences, there are differences between the mismatched hairpin ends encoded by different SPPV and GTPV isolates. Figure 1B shows the flip and flop hairpin ends in structures predicted by M-fold (Zuker, 2003). The length and sequence of the hairpins varies from isolate to isolate with SPPV isolates AG and SAU incorporating an extra 30-33 nucleotides and an extra mismatched loop (Figure 1B). It is apparent that the close relationship detected by a genome-wide comparison of these two isolates (see below) extends to the telomeres. We were unable to obtain sufficient read depth to determine the sequence of the CRS and hairpin ends in LSDV isolate KE. However, some reported LSDV sequences (e.g. KSGP 0240 and Saratov) extend far enough towards the ends of the genomes that a blastn search with the GTPV sequence detected a CRS element (5'-TTTTATAGGCTTAAAAAAAAGTA-3') nearly identical to the ones found in SPPV and GTPV. None of the reported LSDV sequences extend to the hairpin ends.

Relationship between capripoxviruses. The purpose of this work was to gain a better understanding of the genome sequence differences between vaccine and wild-type capripoxvirus isolates. There have been many LSDV isolates sequenced to date, but fewer GTPV and SPPV isolates. Figure 2 shows the results of a k-mer based cluster analysis, which illustrates the relationship between the 13 newly added genomes (in red) and 23 other genomes available on GenBank as at January 2019. This unbiased clustering method is accurate, computationally very efficient, and avoids errors that are introduced through the multiple alignment of long sequences (Deraspe et al., 2017).

This analysis clusters the viruses into the three well-recognized and clearly delineated capripoxvirus species groupings. Note, a strain from Saudi Arabia that was originally described as a GTPV is clearly a SPPV strain by this analysis and will be referred to as a SPPV in this document. Whether this was originally due to SPPV causing an infection in a goat, or some mishap subsequent to virus isolation, is unknown. Two of the new GTPV genomes from Vietnam and India clustered with two Chinese strains [AV41 (MH381810) and FZ (KC951854)] providing further support for the proposal that there are Asian and Middle Eastern GTPV lineages (Tuppurainen et al., 2017), which are not apparent with SPPV or LSDV. It is also

notable that GTPV strains in subgroup 2.3 (Figure 2) (Yemen, Sudan and Oman) infect both sheep and goats (Hajer, Abbas, & Abu Samra, 1988; Kitching, McGrane, & Taylor, 1986), suggesting a common difference that affects host range. In the LSDV group, it is interesting that most of the vaccine strains form a subgroup (SG1.1) distinct from field strains (SG1.2). However, the history of these strains is difficult to trace, and in all probability these vaccines simply share a common ancestry with the Neethling 1959 vaccine (AF409138). The same history may be operating to create the cluster in GTPV subgroup SG2.2.

Differentiating features among capripoxvirus isolates. There is general consensus that poxviruses typically evolve through gene loss and a subsequent narrowing of host range. Among the capripoxviruses these new genome sequences provide enough exemplars to demonstrate that the same pattern of gene loss is also occurring in the telomeres of SPPV, GTPV and LSDV. Figure 3A compares the genes encoded in the first ~20,000 bp at the left end of these viruses. All LSDV encode five genes that have been disrupted by accumulated mutations in both GTPV and SPPV. Despite the loss of function there is little change in sequence length suggesting evolutionary constraints perhaps associated with genome packaging into the virus particle that requires a genome size of close to 150 kbp. Two of these five genes lost have predicted functions. One may serve as an IL-1 receptor decoy (ORF_013). The second is a homolog of vaccinia F11L (ORF_026), which has been shown to have a role in promoting virus exit by inhibiting Rho signalling and disrupting the cortical actin layer (Arakawa, Cordeiro, Schleich, Newsome, & Way, 2007; Cordeiro et al., 2009; Valderrama, Cordeiro, Schleich, Frischknecht, & Way, 2006). When vaccinia F11L was introduced into myxoma virus (which also lacks an F11L homolog), it enhanced virus growth and spread both *in vitro* and *in vivo* (Irwin & Evans, 2012; Irwin, Favis, Agopsowicz, Hitt, & Evans, 2013). The F11L homolog function has been lost from all known lines of SPPV and GTPV, and while it is intact in most LSDV strains, a few (e.g. Evros) also encode mutant homologs. The third lost gene (ORF_009) resembles a C-terminal portion of vaccinia N2L (25-30% protein sequence identity), a gene that encodes an IRF-3 inhibitor (Ferguson et al., 2013).

A similar pattern of gene function loss is seen in the right end of the genome (Figure 3B) where both SPPV and GTPV have a disrupted version of a LSDV gene encoding a 20.4 kDa protein of unknown function (ORF_132). The penultimate LSDV genes encoded in the left and

right terminal inverted repeats (ORF_155) have also been lost. ORF_138 (encoding an OX-2-like protein) is remarkable due to the apparent lower conservation of this gene across the genus. Most SPPV and GTPV “housekeeping” genes share >98% amino acid sequence identity with their LSDV homologs, while this is reduced to 84% identity for ORF_138 due to indel and frameshift mutations that alter the C-termini of the three families of proteins (Table S1). The function of OX-2/CD200 family proteins are dependent upon their membrane localization and a specific interaction with the CD200R receptor (Cameron, Barrett, Liu, Lucas, & McFadden, 2005), and it is noteworthy that despite these alterations the predicted trans-membrane topology and the more broadly conserved central core of the proteins are conserved.

Other genes at the right end of the genome may be involved in host range, in particular host range differences between SPPV and GTPV. The genomes of five out of eight wild-type GTPV strains have an intact ORF_136 whereas the ortholog of this gene in SPPV is interrupted. ORF_136 encodes a protein that belongs to a large family of poxvirus-encoded Toll/IL-1 receptors. Even with the loss of this gene and SPPV_013, SPPV still appears to encode at least two genes with products linked to IL-1-regulated inflammatory signalling pathways.

Genetic variation within GTPV and SPPV isolates. On inspecting Figure 2, it is apparent that much more diversity is detected in GTPV isolates than among SPPV strains. To explore this question further we used “Base-by-Base” (Brodie et al., 2004) to compare the new and previously reported genome sequences (Figure 4) as it offers a better way to visualize the relationships within and between the capripoxviruses. For the GTPV isolates we used strain PL (AY077835) as the reference and compared it to an alignment composed of the six new sequences plus three published GTPV isolates (Figure 4, upper panel). The pattern of mutations across the genomes is suggestive of evolution through random sequence drift with no evidence of recombination. The high degree of sequence variation previously reported for the FZ-China strain (Zeng et al., 2014) reflects the fact that it belongs to a distinct Asian GTPV cluster (Figure 2, SG2.1) thus far composed of FZ-China and isolates from India and Vietnam.

The same method was used to compare the SPPV genomes using SPPV isolate Turkey as the reference genome. The visual image reinforces the observation from Figure 2 that there is much less genetic diversity present in this collection of African, Middle Eastern, and Chinese SPPV isolates than seen in GTPV isolates (Figure 4). The red colour coding in Figure 4 also

clearly illustrates the two unique deletions that map next to the ITR boundaries in SPPV isolate AG and a related SAU vaccine isolate. This region is shown in greater detail in Figure 4 (lower panel). These mutations truncate or delete genes 003, 005, and 006, which encode a putative T4-like apoptosis regulator, IL-10 like molecule, and IL-1 β inhibitor, respectively.

Vaccine-linked mutations: ORF 134 (B22R). Examining the sequence differences between the new and previously published field and vaccine isolates allows inference of genes that may have a role in capripoxvirus virulence. The newly sequenced KE-LSDV isolate is very closely related to LSDV isolates NI-2490 and KSGP0240. The latter strain is also known as “Kenyacac” (Figure 2 and Table S1). Interestingly there is really only one significant difference between the three genomes in that LSDV_134 is disrupted by a +1-frameshift mutation in Kenyacac (A₇ to A₈ at residue K₉₃₃). A +1-frameshift mutation also disrupts LSDV_134 in vaccine isolates LW1959 and OBP (A₇ to A₈ at K₇₁₉). Extending this analysis to GTPV reveals a mutation in GTPV_134 in vaccine isolate G20-LKV. This isolate is closely related to the PL, Gorgon vaccine, and the new TU-GTPV isolate (Table S1). In this case, a +1 frameshift at K₇₀₂ has disrupted the gene, at the same hotspot responsible for the mutations in LSDV isolates LW1959 and OBP. SPPV_134 is also disrupted by a 14 nt deletion in the AG and SAU vaccines.

The protein encoded by ORF_134 shows some similarity (32% identity) to the C-terminal half of the B22R (variola) superfamily. Inactivation of the monkeypox B22R family protein, MPXV197, produces an attenuated virus and the function of the protein is associated with inhibition of T-cell responses (Alzhanova et al., 2014). At least two other poxvirus gene families have been identified that modulate T-cell responses. These are homologs of cowpox (strain Brighton Red) genes CPX012 and CPX203, which inhibit peptide translocation and MHC-I endoplasmic reticulum transport, respectively. All of the capripoxviruses still appear to encode intact homologs of CPX203 (ORF_003), except for SPPV strains AG and SAU (Figure 4). CPX012 is small (69 aa) and no capripoxvirus homolog could be confidently identified by similarity.

Vaccine linked mutations: ORFs 019, 144 and 145. Three other genes were also commonly mutated in vaccine *versus* field isolates. ORF_144 encodes a BTB/POZ domain, cullin binding site, and four C-terminal kelch repeats. It is probably an ortholog of vaccinia virus VV-Cop-

A55R. ORF_145 encodes four ankyrin repeats and a C-terminal PRANC/F-box domain and is likely an ortholog of VV-Cop-B4R. The function of these vaccinia virus genes has been somewhat enigmatic but they are generally understood to promote ubiquitination and degradation of virus and host proteins through interactions mediated by the kelch and ankyrin repeats (B. Liu et al., 2018; R. Liu & Moss, 2018; Sonnberg, Seet, Pawson, Fleming, & Mercer, 2008). ORF_019 encodes a second BTB/kelch protein resembling ORF_144 and is probably an ortholog of VV-Cop-F3L. Inactivation of ORF_019 was observed in some LSDV vaccines.

Reviewing Table S1, all of the related LSDV vaccines (LW1959, Herbivac, OBP, and Cro2016) bear +1 frameshift mutations (T_2 -to- T_3 at S_{256}) in ORF_144 (summarized in Table 3). All of these vaccine isolates also contain mutations in LSDV_019, although some LSDV field strains encode other mutant forms of LSDV_019. The pattern seen in LSDV extends to other capripoxvirus isolates. For example, with the GTPV Gorgon vaccine isolate a 1.6 kbp deletion that spans the 3' end of GTPV_144 and the 5'-end of GTPV_145 appears to inactivate both genes. In GTPV isolate G20-LKV both GTPV_144 and GTPV_145 are intact, but here GTPV_019 is disrupted by a +1 frameshift at F_{226} , again in an A-rich (A_9) sequence (Table 3). SPPV_019 has been shown to be required for virulence after intranasal and intradermal inoculation (Balinsky et al., 2007). Finally, several SPPV strains have an inactivated SPPV_145, including three vaccine isolates (TU, NK, and PE). Interestingly, a sequence difference in the PE genome sequence causes a SPPV_144-SPPV_145 gene fusion, although an in-frame stop codon prevents translation of most of SPPV_145.

Other vaccine-associated mutations. Other genes are inactivated in some but not all vaccine isolates (Kara et al., 2003). The four related LSDV vaccines LW1959, Herbivac, OBP, and Cro2016 all have inactivated ORFs 087 and 131. In these isolates ORF_087 encodes a truncated protein that is likely to be non-functional due to the loss of ~50 aa from the C-terminus. ORF_087 encodes one of the two capripoxvirus proteins with mutT motifs. These proteins catalyze mRNA decapping reactions. Vaccinia virus encodes one gene that is expressed early and one late in infection (Parrish, Resch, & Moss, 2007). LSDV_087 appears to be a homolog of the late-expressed gene (VV-COP-D10R). LSDV_131 encodes a homolog of a catalytically inactive poxvirus Cu-Zn superoxide dismutase. The leporipoxvirus homologs of this protein

modulate superoxide signalling and are also major capsid components that affect virus sensitivity to thermal stress (Teoh, Turner, & Evans, 2005; Zachertowska, Brewer, & Evans, 2004).

Capripoxvirus host range genes. Poxviruses encode an array of additional proteins that interfere with processes like cytokine signalling, apoptosis, and cytotoxic T lymphocyte activation (Epperson, Lee, & Fremont, 2012; Haller, Peng, McFadden, & Rothenburg, 2014; Kvansakul, Caria, & Hinds, 2017; Oliveira, Rodrigues, Lima, Drumond, & Abrahao, 2017; Smith et al., 2013; Smith, Talbot-Cooper, & Lu, 2018; Veyer, Carrara, Maluquer de Motes, & Smith, 2017). Indeed, many commonly recognized poxvirus virulence/host range factors (e.g. K1L, K3L, E3L, serpin, p28/N1R, C7L/M063R, B5R, and T4) have capripoxvirus homologs (Bratke, McLysaght, & Rothenburg, 2013; Haller et al., 2014) and even the missing factors appear to be complemented by genes targeting other elements of the pathway. For example, capripoxviruses have no M013L homolog (Bratke et al., 2013), but still encode inhibitors of downstream effectors in the same targeted inflammatory pathways (e.g. IL-1 β). The new sequences provided an opportunity to test whether there were any notable differences in these genes that might better explain the differences in host range and virulence in vaccine and field isolates.

In general, these capripoxvirus genes individually clustered in a manner that recapitulated the host-range clusters detected using whole genome data. However, the highest-level branches sometimes grouped SPPV with LSDV, or GTPV with LSDV, but never clustered SPPV with GTPV as is so clearly seen using whole genome data (compare Figure 2 and Figure 5). For example, capripoxviruses encode proteins belonging to the K3L (ORF_014) and E3L (ORF_034) families of inhibitors that modulate PKR activity. K3 binds to and inhibits the activated and phosphorylated form of PKR by mimicking the structure of its natural target eIF2 α (Carroll, Elroy-Stein, Moss, & Jagus, 1993; Kawagishi-Kobayashi, Cao, Lu, Ozato, & Dever, 2000; Kawagishi-Kobayashi, Silverman, Ung, & Dever, 1997). All of the capripoxviruses encode K3 homologs, all the proteins encode a conserved KGYID motif required to inhibit PKR (Kawagishi-Kobayashi et al., 1997), and all of the genes clustered in clearly delineated SPPV, GTPV, and LSDV subgroups although, in this case, the GTPV genes seemed to form the out group (Figure 5). A recent report showed that while the GTPV K3 homolog could inhibit both goat and sheep PKR, the SPPV K3 homolog inhibited sheep PKR better than goat PKR (Zhao et

al., 2019). The fact that ORF_014 operates and evolves along host-specific lines should be considered in designing pan-species vaccines.

All capripoxviruses also encode E3L homologs (ORF_034). These proteins act upstream of K3 by binding to the duplex RNA that would otherwise induce PKR activation. As with K3, the SPPV, LSDV and GTPV genes sub-clustered in the same subgroups determined by the host range and with GTPV as the out group (Figure 5). The amino acid sequence of ORF_034 is highly conserved between different capripoxvirus isolates, with no difference in sequence across much of the C-terminal dsRNA binding domain nor mutations in residues required for RNA binding (Ho & Shuman, 1996). Despite this high degree of sequence conservation within the capripoxvirus genus, SPPV_034 has still diverged sufficiently from other orthologs that the gene could not complement an orthopoxvirus E3L deletion in HeLa cells (Myskiw et al., 2011).

The serpins comprise a superfamily of serine protease inhibitors that regulate many biological processes. Some poxvirus families encode up to three serpins, the capripoxviruses encode just one (ORF_149). An analysis of the relatedness between these genes (Figure 5) also detects clusters appropriate to the host range (Figure 2). Exactly what role(s) is/are served by ORF_149 aren't known, but clues can be obtained by inspecting the sequence around the C-terminal reactive centre loop (RCL). The RCL is a primary determinant of target specificity. The sequence around the ORF_149 RCL more closely resembles the rabbitpox and cowpox Serpin-2 RCL and suggests that ORF_149 belongs to the orthopoxvirus SPI-2 family of protease inhibitors (Figure 6) (Bratke et al., 2013; Hood, Huntington, & Gettins, 1994; Moon, Turner, & Moyer, 1999). The remainder of the RCL is highly conserved among capripoxviruses except for an Ala-to-Thr substitution at position P14 and a Cys-to-Phe substitution at site P4' in SPPV compared to GTPV and LSDV (Figure 6). The two SPPV amino acid substitutions hint at there being some difference in the protease targets. This domain is truncated in SPPV strain AG although the related SAU vaccine strain still encodes an intact protein.

The capripoxviruses also encode homologs of K1L (ORF_12), C7L (ORF_067), and B5R (ORF_141). K1 blocks I κ B α degradation (Shisler & Jin, 2004), C7 antagonizes antiviral activities regulated by SAMD9 and IRF-1 (Meng et al., 2012; Sivan, Ormanoglu, Buehler, Martin, & Moss, 2015), and B5 promotes virus exit (Engelstad, Howard, & Smith, 1992). All three gene families also sub-cluster in a manner coincident with the host range (Figure 5). Besides B5R/ORF_141, capripoxviruses apparently encode all the other proteins required for

extracellular envelope virus assembly (F12/ORF_027, F13L/ORF_028, A27/ORF_117, A33R/ORF_122, A34R/ORF_123, and B5R/ORF_141). An A36R homolog is not detected, but its function may be dispensable (Roberts & Smith, 2008) and, as noted above, the F11L homolog has been lost in SPPV and GTPV.

Of these genes only two families appear to fall into clusters in some disagreement with whole genome analyses. The M11L/F1L family of genes (ORF_017) encode mitochondrion localized anti-apoptotic proteins that fold into structures resembling Bcl-2 (Bratke et al., 2013; Kvansakul et al., 2007; Wasilenko, Stewart, Meyers, & Barry, 2003). In this case many of the LSDV vaccine isolates aren't incorporated into a cluster composed of just LSDV strains. Elsewhere, the capripoxvirus inverted terminal repeats encode duplicated homologs of the T4 family of ER-localized apoptosis inhibitors (ORF_003/154). These genes are of interest as the myxoma virus homolog (M-T4) promotes virus dissemination *via* lymphocyte trafficking (Barry et al., 1997), while the cowpox virus homolog (CPXV203) inhibits MHC-Class I transport (Byun, Wang, Pak, Hansen, & Yokoyama, 2007; McCoy, Wang, Yokoyama, Hansen, & Fremont, 2012). MT-4 and CPXV203 encode the C-terminal ER-targeting motifs RDEL and KTEL, respectively, while capripoxviruses encode a C-terminal HNEL motif. Whether it can also serve as another ER trafficking signal has yet to be established (Nelson, Epperson, Singh, Elliott, & Fremont, 2015). It is curious that while the SPPV genes cluster together, the GTPV and LSDV genes form clusters poorly aligned with host range (Figure 5). Moreover, a group composed of the GTPV strains that also infect sheep (YE, SU, and OM) occupy a cluster distinct from the other LSDV and GTPV strains. Mutations have inactivated the gene in the two related SPPV strains AG and SAU vaccine, but otherwise ORF_003/154 is conserved across many field and vaccine isolates.

CONCLUSIONS

Capripoxviruses cause significant economic losses for the world's livestock industries and devastate the lives of small farm holders (Hopker et al., 2019). Genetically stable vaccines that can produce long-term immunity against all three capripoxvirus members, and in the different host species, would be an asset to halt the continued spread of these agents.

This study further supports the suggestion that capripoxviruses have speciated through a process of gene loss from an ancestral LSDV-like virus (Tulman et al., 2002). The source of the

ancestral LSDV-like virus is unknown and is likely not the current lumpy skin disease virus circulating today, since lumpy skin disease was only discovered many centuries after sheeppox and goatpox, in a region of Africa free of both SPPV and GTPV. The loss of ORFs 009, 013, 026, 132, and 002/155 appears to have produced the SPPV/GTPV lineage, and the subsequent loss of ORF_136 has likely played a role in the evolution of SPPV isolates. The greater degree of genetic diversity (Figure 2), and broader geographical range of GTPV versus SPPV isolates, also supports the hypothesis that SPPV evolved relatively recently from a still uncharacterized (or possibly extinct) GTPV lineage.

If one compares the mutations that differentiate vaccine from field isolates one also detects a number of common threads, although care should be taken not to confuse mutations arising independently with those shared through common descent. Moreover, some older automated sequencing methods could not accurately count nucleotides in simple repeats (e.g. $>A_{5-6}$) and thus we cannot exclude the possibility that a few frameshift mutations are sequencing errors compounded by assemble-to-reference methods. Nevertheless, a common pattern is seen where large proteins are often disrupted by frameshift and other mutations and this includes genes encoding ORF_019, ORF_134, ORF_144, and ORF_145. These are primarily proteins encoding kelch and ankyrin repeats (Table 3). It had previously been noted that ORF_019 was mutated in GTPV and LSDV and after deleting SPPV_019 showed that this mutation also attenuates SPPV (Balinsky et al., 2007). Some of these mutations are detected in several different capripoxviruses, for example ORF_134 (encoding a B22R homolog) is mutated in LSDV, SPPV, and GTPV strains and would be a highly desirable feature if the mutation enhanced vaccine responses. Other more idiosyncratic mutations are detected in genes that encode mutT and superoxide dismutase homologs in LSDV strains and in SPPV_148, which also encodes a protein with ankyrin repeats.

Most of these vaccine strains were produced by classical methods and our analyses do suggest some cautionary notes. LSDV vaccine strains LW1959, Herbivac, OBP, and Cro2016 are all closely related judging by the pattern of shared mutations (Table S1) and yet it is striking that Herbivac alone encodes a full-length LSDV_134 gene. The most parsimonious explanation is that this strain has suffered a reversion mutation to restore LSDV_134 function (Mathijs et al., 2017). This family of LSDV vaccine isolates also all encode a deletion in the superoxide dismutase homolog LSDV_131. Its homolog has been shown to play a role in stabilizing

leporipoxvirus particles against thermal stress (Zachertowska et al., 2004) and such a mutation may not be desirable in a vaccine where a cold chain cannot be guaranteed. Many spontaneous mutations in poxviruses can be traced to small repeats and duplications (Qin, Upton, Hazes, & Evans, 2011), and the same pattern is detected in these vaccine isolates especially at A/T-rich regions. These observations highlight the need to use molecular approaches to introduce more well-defined and stable mutations into the next generation of capripoxvirus vaccines.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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FIGURE LEGENDS

Figure 1. Alignment of sequences encoding the hairpin ends of GTPV and SPPV genomes.

The Illumina sequencing reads encoding the ends of each genome were retrieved using a poly·dC template attached to the 5'-end of a reference genome and encompassing the highly conserved concatemer-resolution site (CRS). This retrieved a series of reads of varying length spanning the flip and flop ends of each genome (Noyce et al., 2018). (A) Alignment of sequences extending from one CRS on the left end to its nucleotide complement on the right end. The Abu Gharib (AG-SPPV) and Saudi Arabia (SAU-Vac) strains are closely related and encode 30-33 more nucleotides in the middle of the imperfect inverted repeat symmetry (arrows). (B) Proposed folding patterns. How these structures might base-pair was modeled using the M-fold server with default parameters (Zuker, 2003). The CRS elements are colour coded in green and the hairpins and loops are shown in black. Both the flip and flop forms are shown here, one imperfect duplex is complementary to the other.

Figure 2. Genome-based phenetic analysis of capripoxvirus isolates. The k-mer method (Deraspe et al., 2017) and CLC genomics were used to estimate the degree of similarity between 36 complete genomes. The three well-established LSDV, GTPV, and SPPV groupings are clearly delineated, and within the GTPV group some further geographical distinctions are apparent (subgroups 2.1, 2.2, and 2.3). LSDV Saratov is probably a recombinant isolate (Sprygin et al., 2018). Also clearly apparent are a cluster of closely related LSDV vaccine strains (subgroup 1.1) and the more limited genetic diversity among these SPPV strains. Essentially identical bootstrap consensus trees, inferred from 100 replicates, were also obtained using the maximum likelihood method in MEGA7. The new SPPV, GTPV, and LSDV sequences are highlighted in red.

Figure 3. Patterns of gene loss in the left and right ends of capripoxvirus genomes. (A) The left ends of the LSDV, GTPV, and SPPV genomes encompassing ORF_001 to ORF_027 (~20 kbp). The genes that appear to have been commonly lost through disruptive mutations in the GTPV and SPPV lineages are highlighted in green. (B) The right end of the LSDV, GTPV, and SPPV genomes encompassing ORF_128 to ORF_156 (~30 kbp). The left and right inverted

terminal repeats (LITR and RITR, respectively) are marked in yellow and also highlighted are the ankyrin repeat and kelch-like proteins (in red) and the B22R homologs (in blue). These proteins are commonly disrupted by mutations in vaccine isolates and are discussed elsewhere.

Figure 4. Sequence variation among different GTPV and SPPV isolates. The two upper panels show whole genome alignments of ten different GTPV and eleven different SPPV isolates. The sequences were aligned using LAGAN and edited with Base-by-Base. Each genome in each stack was compared to the genome at the top of that stack (i.e. PL-GTPV and TU-SPPV). Any differences between each two pairs of viruses were color-coded to indicate insertions (green), substitutions (blue), and deletions (red). The figure in the lower part of the panel shows the effects of the many deletions on the genes encoded in left end of the genome in SPPV isolates AG-SPPV and SAU-Vac. SPPV isolate Pendick served as a reference genome. Only fragmented remnants remain of genes encoding a T4-like protein (ORF_003), an IL-10 like protein (ORF_005), and a putative IL-1 β inhibitor (ORF_006). Also shown are the location of the putative concatemer resolution sequences (CRS) and the left-hand terminal inverted repeats.

Figure 5. Phenetic relationships within capripoxvirus host range/virulence genes. BLASTN searches were used to retrieve homologous gene sequences from the NCBI databases and these libraries were supplemented with the newly obtained LSDV, GTPV, and SPPV gene sequence data. The alignment file was used to generate a phenetic tree using CLC Genomics. The LSDV, GTPV, and SPPV gene clusters are highlighted in green, grey, and cyan, respectively. Only schematic tree topologies are shown and the branch lengths are not drawn to scale. As a rule, genes cluster in a manner predicted by that virus's host range, but LSDV never forms the out-group in higher level clusters.

Figure 6. Amino acid sequences surrounding the reactive center loop (RCL) in representative LSDV, GTPV, and SPPV serpin proteins (ORF_149). The homologous regions in the cowpox (Brighton Red) and rabbitpox (Utrecht) virus SPI-1 and SPI-2 serpins are shown for reference purposes. The LSDV and GTPV proteins likely share a similar protease target, but possibly not the same protease targeted by SPPV_149 judging by substitutions at the

P14 and P4' sites. GenBank entries for LSDV NI-2490, GTPV Pellor, and SPPV India are AF325528, AY077835, and AXX92162.1, respectively.

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Table 1. Capripoxviruses sequenced for this study

GenBank ID	Isolate name	Abbreviated name	Origin	Year	Virus type	Field or vaccine	Genome (bp)	ITR [†] (bp)	Note	Reference
MN072619	Kenyan LSDV	KE-LSDV	Kenya	1958	LSDV	Field	150,329	2196	Similar to NI-2490 [‡]	
MN072621	Vietnam GTPV	VI-GTPV	Vietnam	2005	GTPV	Field	150,289	2370	Goat lung	(1)
MN072620	India GTPV	IN-GTPV	India (Chiplima)	1983	GTPV	Field	150,381	2391	Goat skin	(2-4)
MN072622	Turkey GTPV	TU-GTPV	Turkey	U [§]	GTPV	Field	150,001	2353	U	
MN072625	Yemen GTPV	YE-GTPV	Yemen (Sana'a)	1983	GTPV	Field	150,450	2360	Goat lung	(1-5)
MN072623	Oman GTPV	OM-GTPV	Oman (Sohar)	1984	GTPV	Field	150,404	2266	Goat skin	(4-6)
MN072624	Sudan GTPV	SU-GTPV	Sudan (Nyala)	1983	GTPV	Field	150,393	2334	Goat skin	(3, 4)
MN072627	Saudi Arabia vaccine	SAU-Vac	Saudi Arabia	U	SPPV	Vaccine	148,753	2106	Rhone Merieux origin?	RPK (pers. com)
MN072626	Abu Gharib SPPV	AG-SPPV	Iraq	U	SPPV	Vaccine?	148,630	2086	Likely a vaccine	RPK (pers.com)
MN072631	Turkey SPPV vaccine	TU-Vac	Turkey	U	SPPV	Vaccine	150,218	2405	U	
MN072630	Saudi Arabia SPPV [¶]	SAU-SPPV	Saudi Arabia	U	SPPV	Field	150,212	2403	U	
MN072629	Pendik SPPV	PE-SPPV	Turkey	1983	SPPV	Vaccine?	150,353	2402	Likely a vaccine	RPK (pers. com.)
MN072628	Nigeria SPPV	NIG-SPPV	Nigeria	1977	SPPV	Field	149,956	2257	Sheep skin	(2, 4, 7)

Footnotes:

[†] Inverted terminal repeat (length as assembled through sequencing)

[‡] This isolate very closely resembles LSDV strain NI-2490 (Neethling) originally isolated in Kenya in 1958

[§] U = unknown

[¶] Although originally labelled a GTPV, sequencing showed that this strain is a SPPV.

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Table 2. Other sequence data employed in this study.

GenBank ID	Full name	Abbreviated name	Virus type	Field or vaccine	Reference
MG972412	Cro2016 LSDV	Cro2016 vaccine	LSDV	Vaccine	(Lojkic, Simic, Kresic, & Bedekovic, 2018)
KX764645	Neethling LSD vaccine-OBP	OBP vaccine	LSDV	Vaccine	(Mathijs, Vandenbussche, Haegeman, King, et al., 2016)
KX764644	Neethling Herbivax vaccine	Herbivax vaccine	LSDV	Vaccine	(Mathijs, Vandenbussche, Haegeman, King, et al., 2016)
KX764643	SIS Lumpyvax vaccine	SIS vaccine	LSDV	Vaccine	(Mathijs, Vandenbussche, Haegeman, King, et al., 2016)
AF409138	Neethling vaccine 1959	LW1959 vaccine	LSDV	Vaccine	(Kara et al., 2003)
MH64667	Russia/Saratov 2017 LSDV	Saratov LSDV	LSDV	Vaccine	(Sprygin et al., 2018)
KY829023	Evros/GR/15 LSDV	Evros/GR/15	LSDV	Field	(Agianniotaki et al., 2017)
KY702007	SERBIA/Bujanovac/2016 LSDV	Bujanovac2016	LSDV	Field	(Toplak et al., 2017)
MH893760	Russia/Dagestan/2015	Dagestan2015	LSDV	Field	(Sprygin et al., 2019)
KX894508	155920/2012 LSDV	155920/2012	LSDV	Field	
AF409137	Neethling Warmbaths LW	NW-LW	LSDV	Field	(Kara et al., 2003)
KX683219	KSGP 0240 LSDV	KSGP0240	LSDV	Vaccine	
AF325528	Neethling 2490 LSDV	NI-2490	LSDV	Field	(Tulman et al., 2001)
KC951854	China_FZ GTPV	FZ-China	GTPV	Field	(Zeng et al., 2014)
MH381810	China_AV41 GTPV	AV41-China	GTPV	Field	
AY077836	G20-LKV GTPV	GV-vaccine	GTPV	Vaccine	(Tulman et al., 2002)
AY077835	Pellor GTPV	PL-GTPV	GTPV	Field	(Tulman et al., 2002)
KX576657	Gorgan GTPV	Gorgan vaccine	GTPV	Vaccine	(Mathijs, Vandenbussche, Haegeman, Al-Majali, et al., 2016)
KT438551	GL-SPPV	GL-SPPV	SPPV	Field	

KT438550	GH-SPPV	GH-SPPV	SPPV	Field	
AY077834	Niskhi SPPV	NK-vaccine	SPPV	Vaccine	(Tulman et al., 2002)
AY077833	SPPV-A	SA-SPPV	SPPV	Field	(Tulman et al., 2002)
AY077832	Turkey SPPV TU-V02127	TU-SPPV	SPPV	Field	(Tulman et al., 2002)

Table 3. Patterns of mutation in selected Capripoxvirus genes. The numbers report the degree of identity, in percentage, between the reference LSD/Kenya gene and the homolog in each indicated strain. Only low (<90%) GATU scores are shown, which are typically indicative of truncated or fragmented genes. Gray hatching indicates vaccines.

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Subgroup	Isolate	ankyrin repeat proteins					kelch-like proteins			B22R
		12	145	147	148	152	19	144	151	
1.1	Cro2016 vaccine						76.8	50.5		61.2
1.1	OBP vaccine						76.8	50.5		61.2
1.1	Herbivac vaccine						76.8	50.5		
1.1	LW_1959 vaccine						76.8	50.5		61.2
1.1	Saratov LSDV						77.2			
1.2	Evros/GR/15						51			
1.2	NW-LW						51			
1.2	KSGP0240									54.5
1.2	NI-2490									
2.1	VI-GTPV									
2.1	FZ-China									
2.1	In-GTPV									
2.2	GV-Vaccine						48.7			60.9
2.2	PL-GTPV									
2.2	Gorgan vaccine		57.6					61.1		
2.2	TU-GTPV									
2.3	YE-GTPV									
2.3	OM-GTPV									
2.3	SU-GTPV									
3.1	SAU-Vac					55.6				37.4
3.1	AG-SPPV									37.4
3.2	GL-SPPV									
3.2	GH-SPPV									
3.2	NK-vaccine		57.3		38.3					
3.2	TU-Vac		52.7							
3.2	SA-SPPV									
3.2	SAU-SPPV		52.7							
3.2	PE-SPPV		57.3					86.6		
3.2	TU-SPPV									
3.2	NIG-SPPV									

A. Linear (junction) form

B. M-Fold models

tbed_13322_f1.eps

tbed_13322_f2.eps

A.

KE LSDV

OM GTPV

NIG SPPV

B.

KE LSDV

OM GTPV

NIG SPPV

Goatpox virus

Sheeppox virus

tbed_13322_f4.eps

■ LSDV
■ SPPV
■ GTPV

tbed_13322_f5.eps

ORF_149, ~340 aa

Rabbitpox SPI-1 EYTEASAVTGVFMTNFSMVYRTKVYINHPFMYMIK
Cowpox SPI-1 EYTEASAITGVFMTNFSMVYRTKVYINHPFMYMIK
Rabbitpox SPI-2 EYTEAAAATSVLVADCASTVTNEFCADHPFIYVIR
Cowpox SPI-2 EYTEAAAATCALVADCASTVTNEFCADHPFIYVIR
LSDV NI-2490 EGAELSAASYCCVADCGCNE-KEFIANKPFIFLIK
KE-LSDV EGAELSAASYCCVADCGCNE-KEFIANKPFIFLIK
GTPV Pellor EGAELSAASYCCVADCGCNE-KEFIANKPFIFFIK
IN-GTPV EGAELSAASYCCVADCGCNE-KEFIANKPFIFFIK
TU-GTPV EGAELSAASYCCVADCGCNE-KEFIANKPFIFFIK
SPPV India EGTELSAASYCCVADCGFNE-KEFIANKPFIFLIK
NIG-SPPV EGTELSAASYCCVADCGFNE-KEFIANKPFIFLIK
PE-SPPV EGTELSAASYCCVADCGFNE-KEFIANKPFIFLIK

P14

P1

P4'

P14'

tbed_13322_f6.eps

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