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Genome Annotation and Comparative Genomics of ORF Virus

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Abstract

ORF virus (ORFV), the etiological agent of contagious pustular dermatitis in small ruminants, belongs to members of the genus *Parapoxvirus* of the *Poxviridae*. The genome of the ORFV is dsDNA of 139,962 bp which has about 89% coding region, 63% GC content and codes 130 proteins. There are four unique genes within the genome revealed by homology search of them two posses' strong regulatory region and transmembrane helices. One of the ORF-039 contains signal peptide indicating the possibilities to be secretory protein coding gene. Comparative genomic analysis reveals significant differences in Bovine Papular Stomatitis Virus (BPSV) strain BV-AR02 and ORFV strain OV-SA00, and these may account for differences in host range. Interspecies sequence variability is observed in all functional classes of genes but is the highest in putative virulence/host range genes. Notably, ORFV contains genes which are homologous of Vaccinia virus. Phylogenetic analysis reveals that although divergent, ORFV virus is distinct from other known mammalian cowpox virus. An improved understanding of Parapoxvirus (PPV) biology will permit the engineering of novel vaccine viruses and expression vectors with enhanced efficacy and greater versatility. The novel vaccine will have a significant role in the economy of a country through the control of disease in an economically important and small ruminant caused by ORFV.

Keywords

ORF Virus, Contagious Pustular Dermatitis, Comparative Genomics, GLIMMER, ACT, Uniue Genes, Novel Vaccine

1. Introduction

Genome annotation is the analysis of genome sequence of a particular species which includes all the possible

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analysis of DNA sequence that can be done by computational means. Raw DNA sequence produced by the genome-sequencing projects is taken for the analysis. Analysis and interpretation is carried out which is necessary to extract its biological significance and organize the information into the context of our understanding of biological processes. By genome annotation, an individual gene and its protein (or RNA) product can be predicted by in depth analysis of several gene features, such as ORFs length, % (G + C) content, promoter, polyA tail, homology, etc. The focal point of each such record is the validity of a ORF as a potential functional gene. Moreover, analysis may also include a brief description of the evidence for the assigned or proposed function. Several genome annotation tools are freely available for further analysis of genome sequence. Users can use several individual tools for each task or can use some integrated tools that do many task simultaneously. GLIMMER [1], GeneMark [2], ORF finder [3] and FrameD [4] are well-used tools for predicting Open Reading Frame (ORF). BLAST is the most used homology tool for homology prediction but there are many other options are available, such as MPsrch, PSI-BLAST and WU-BLAST [5]. Artemis, Apollo, JBROWSE, etc. are used for Genome browsers for curation and further annotation [6]. There are also many Genome annotation pipelines available, such as PASA and MAKER, in which several analysis tools are integrated [6]. Users are required to select the tools or pipeline according to their needs that serve best for their purpose

Contagious pustular dermatitis; ORF is a common epitheliotropic viral disease of sheep, goats and wild ruminants and is characterized by the formation of papules, nodules, or vesicles caused by the ORF virus [7]. Humans always posses a high risk due to zoonotic characteristics of this disease and often humans can contract this disorder through direct contact with infected animals by the fomites that carry the ORF virus [8]. Purulent-appearing papule is the major symbol causes locally and generally no systemic symptoms is obseved [3]. Normally it infects the finger, hand, arm, face and even the penis [6]-[9]. ORF virus (ORFV) is an oval and enveloped virus containing dsDNA genome within the genus *Parapoxvirus*, family *Poxviridae* [6]. The genus also includes pseudocowpox virus (PCPV) and bovine papular stomatitis virus (BPSV) in cattle and parapoxvirus (PPV) of red deer in New Zealand [10].

Mechanisms involved in ORFV virulence are not well studied [10]. Several putative virulence genes have been identified, such as the vascular endothelial growth factor homologue, an interleukin (IL-10) homologue, a double-stranded RNA-binding protein, a factor inhibiting the cytokines granulocyte-macrophage colony-stimulating factor and IL-2 [11]-[16]. The whole genome of ORF virus strain OV-IA82 has been sequenced by the Plum Island Animal Disease Center, USA in 2004 [17]. The genome has high 63% GC content with 89% coding region which codes 130 proteins and length is 139,962 bp [17]. The complete genomic sequence available for OV-IA82 enables to deduce the different complex area including other molecular aspects of pathogenesis of this virus in details [18]. In this study, we tried to identify unique genes in ORFV genome and characterize them by Insilco process.

2. Materials and Methods

There are certain kinds of genome annotation tools available for analysis the whole genome, some of which Artemis and Artemis comparison Tools (ACT) has been used extensively for Open Reading Frame (ORF) visualization, editing, determine GC plot as well as retrieve desired nucleotide length within the genome for further analysis [19]. The GLIMMER has been chosen for predicting the genes or ORF in the genome of ORFV based up on its accuracy and flexibility of changing stop and start codons according to the requirement [20] [21]. Potential protein coding ORFs were identified by the following criteria: ORF size larger than 60 amino acid (aa), presence of potential transcriptional start and stop sites, a high GLIMMER score and homology to other known *Parapoxvirus* or cellular ORFs [21]. To find the similarity and homology, sequences of the GLIMMER pedicted protein, coding ORF were compared with the protein databases such as SwissProt, Trembl, UniProt, etc. In this study, among the tools which are available to find the similarity compared to protein databases, the Blast module Blastall, which supports all five Blast programs (blastp, blastn, blastx, tblastn and tblastx) has been chosen for finding the unique genes. Promoter, poly A signal and CpG island were analyzed for each unique genes in order to determine their potentiality as genes. For promoter prediction upstream ~350 bases were subjected within Neural Network Promoter Prediction Tool developed by University of Berkly, USA with a cut off value 0.8 [22]. PolyA signals were determined by polyADQ which is a poly (A) signal search engine developed by Cold Spring Harbor Laboratory, USA [22] [23]. The software CpGIE was downloaded via the website: [24]. The following cutoff values were used to determine the CpG island in a given genomic sequence: ≥200 nt, G + C content 50%,

and an observed: expected CpG ratio 0.6 [25] [26]. To determine the Trans Membrane (TM) domain and peptide signal of the unique genes TMHMM and SignalP 3.0 software was used respectively [27]. The Artemis and Artemis Comparison Tool (ACT), written by Kim Rutherford, Sanger Institute, UK [19] were used for genome analysis and the pair wise comparisons between OV- IA82, VACV and BPSV genomes. For phylogenetics analysis various B2L genes of ORFV were selected on the basis of host specificity such as human, sheep and goats [28] [29]. In order to determine distant relationship BPSV and psedocowpox B2L gene also selected. All the genes sequences were downloaded from gene bank and stored for phylogenetic tree construction as listed in **Table 1**. The nucleotide sequences of diverse ORf viruses and others were aligned using the Bioedit program and Mega 5 software [22]. One thousand bootstrap replicates were subjected to nucleotide sequence distance and neighbor-joining methods, and the consensus phylogenetic tree was drawn.

3. Results

In this study we tried to identify unique genes in ORFV on the basis of the availability of complete genome sequence of OV-IA82 by Insilco process in order to determine the novel vaccine strain. Recent advances in bioinformatics enable us to further analysis of the ORFV genome and the results are given below.

3.1. Coding Potential and Functional Analysis

GLIMMER predicted 130 potential open reading frames which supported previous studies. Like other poxviruses, ORFV genomes contain a large central coding region (ORF 12) bounded by two identical inverted terminal repeat (ITR) regions, (ORF 26 and 48). Homology search by blast revealed four ORFs 039, 116, 124 and 125 as unique genes as no similarity has been found with others rather than with ORFV. Only one ORF (039), previously described for ORFV strains NZ2 and NZ7, is completely located within the ITRs of the genome.

3.2. Regulatory Regions of the Unique Genes

Among the four unique genes, all posses promoter above cut off value 0.9 in the nearer upstream region which is

Table 1. List of genes for phylogenetic analysis with origin and gene bank accession number.

Description	Country of Origin	Species of Origin	Region of Gene	Accession Number
ORF/2009-B2L	Korea	Goat	B2L	GQ328006
Taiping (Human infected)	Taiwan	Goat	B2L	EU327506
ORF-sh	USA	Sheep	B2L	AY424970
NZ-2-1	New Zealand	Sheep	B2L	U06671
India 67/04	India	Sheep	B2L	DQ263305
India 79/04	India	Sheep	B2L	DQ263306
ORF-ca 1	USA	Goat	B2L	AY278208
ORF-mu	USA	Musk ox	B2L	AY424969
ORF-va1	USA	Goat	B2L	AY278209
ORF-ta	USA	Takin	B2L	AY424971
India 59/05	India	Goat	B2L	DQ263304
Nantou	Taiwan	Goat	B2L	DQ904351
Hoping	Taiwan	Goat	B2L	EU935106
PCPV-TQ	NA	Cow	B2L	AY424972
BPSV-RS	NA	Calf	B2L	AY424973

an indication of their translation probabilities. On the contrary, ORF 124 and 125 lack polyA signal as well as CpG island. Besides ORF 039 and 116 have strong promoter as well as CpG island 51 and 52 bp long respectively. ORF 039 has polyA signal in their 6 bp downstream region but ORF 116 lacks polyA signal.

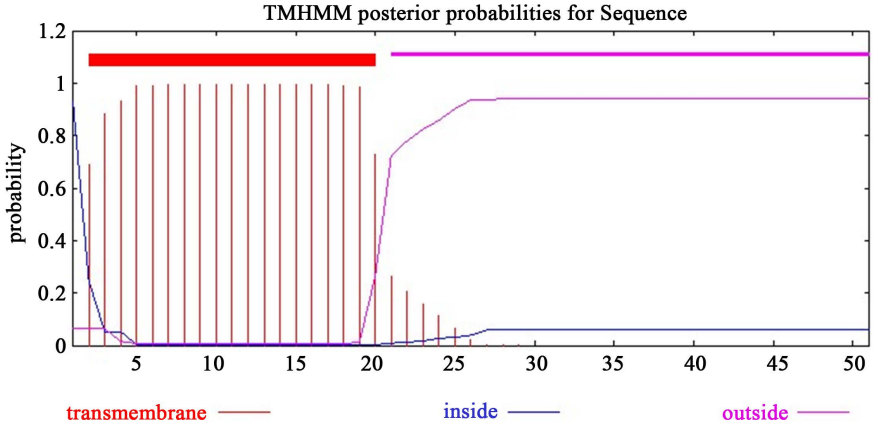
3.3. Transmembrane Domains and Signal Peptides of the Unique Genes

All four unique genes were further evaluated to determine the TM helics. ORF 039 contains TM helices in between 2 - 20 amino acid (aa) in their C-terminal region in which inside helices is in 21 - 51 aa. On the other hand, ORF 116 contains TM helices in between 26 - 48 aa, in which 1 - 28 aa are inside the membrane and 49 - 52 aa are outside the membrane (**Figure 1**).

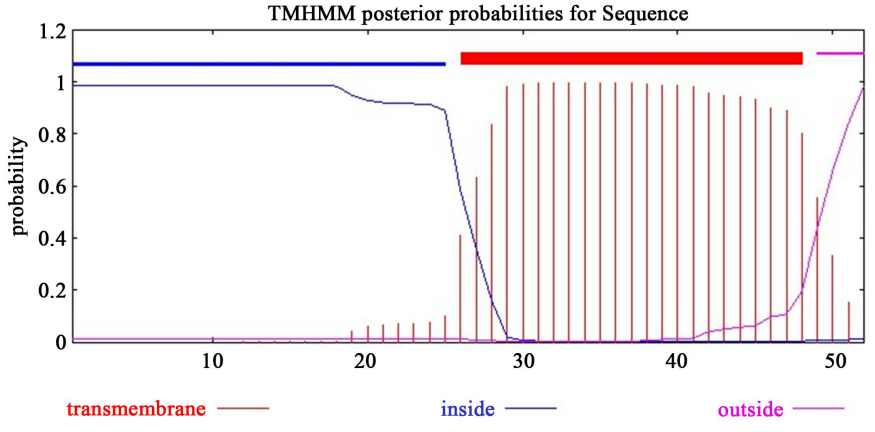
Besides, only ORF 039 was found having peptide signal at 31 and 32 aa with a probability 0.82. For confirmation both the PAM Matrix (PM) algorithm and Hidden Markov Model (HMM) were used (**Figure 2**). Other three ORFs lack signal peptides, by which we can assume that they are not secretory proteins.

3.4. Comparative Genomics

Three genomes of Orf virus (ORFV) strain OV-IA82, bovine papular stomatitis virus (BPSV) strain BV-AR02 and vacccinia virus (VACV) strain VACV-A4L were used for comparative genomics in order to find the unique genes (**Figure 3**).



(a)



(b)

Figure 1. Determination of TM helics. Here, (a) represents the output result of TMHMM for ORF 039 and (b) represents the TMHMM result for ORF 116 in which transmembrane helices are shown in bold red line and inside and outside membrane helices are shown in blue and purple line, respectively.

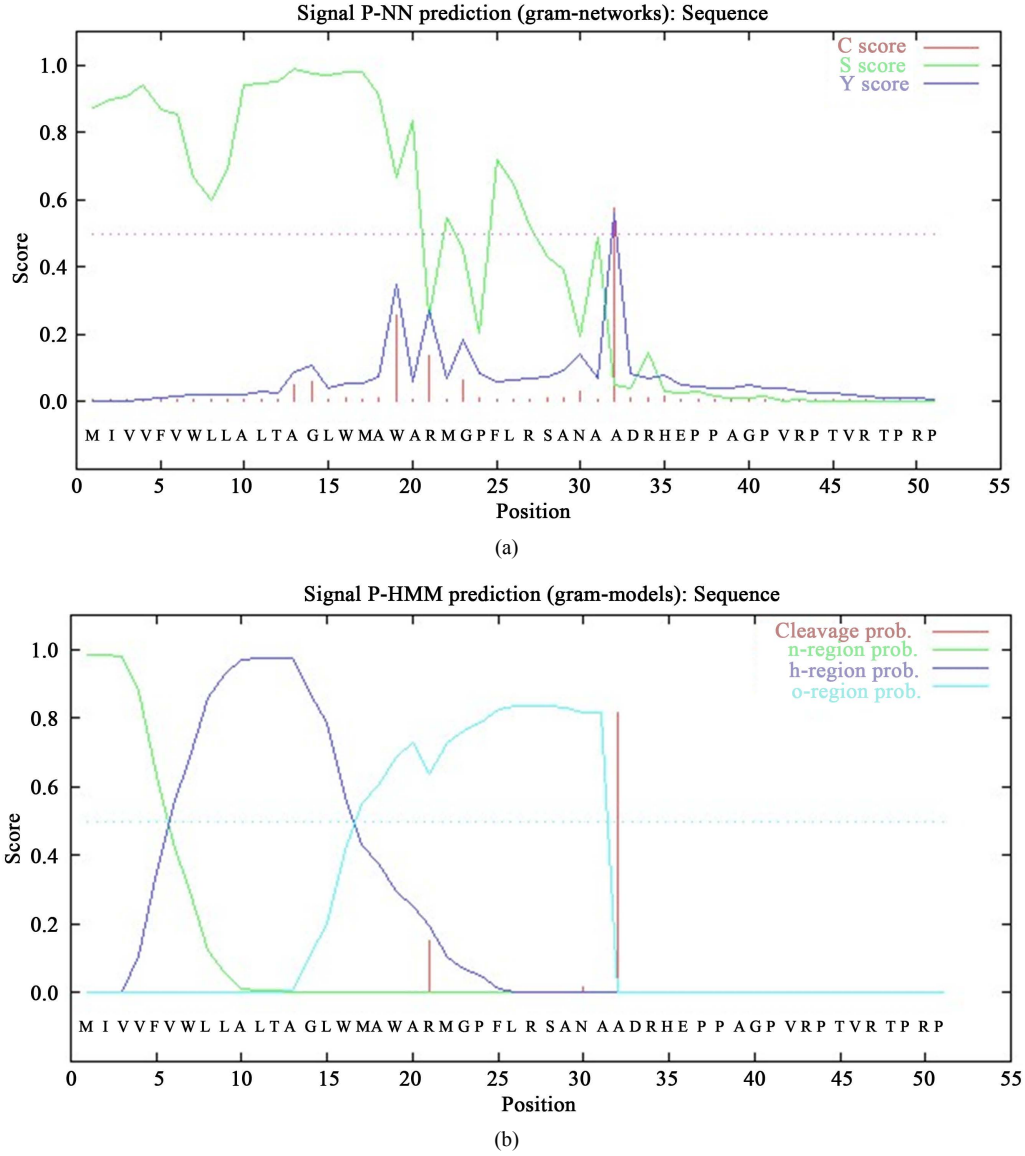
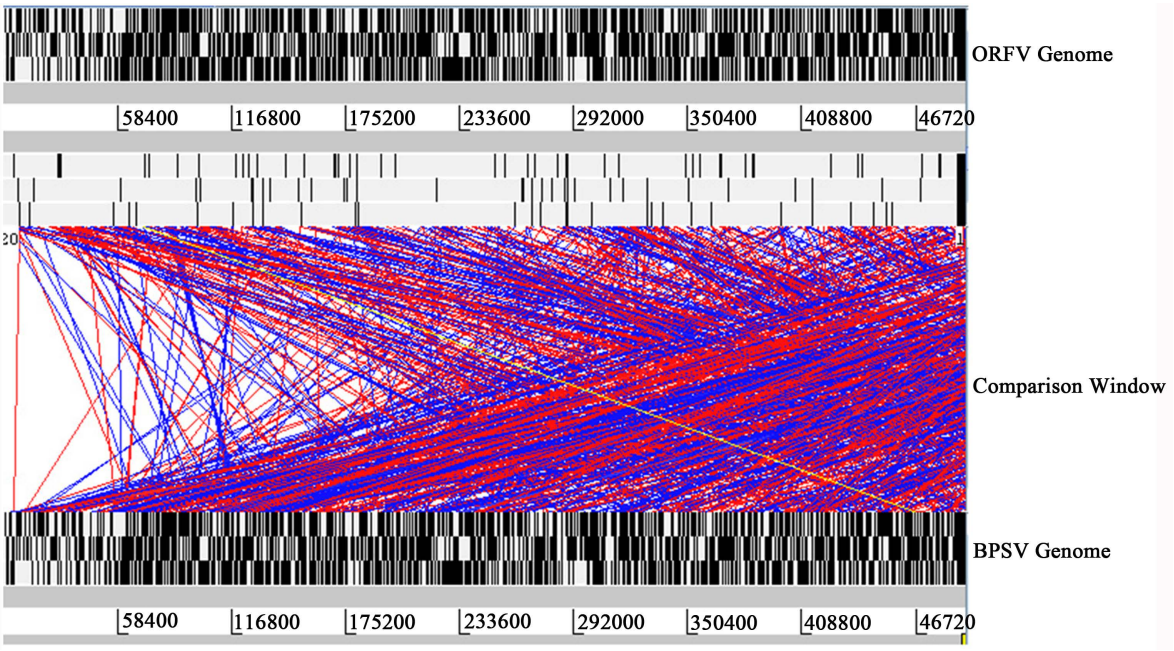


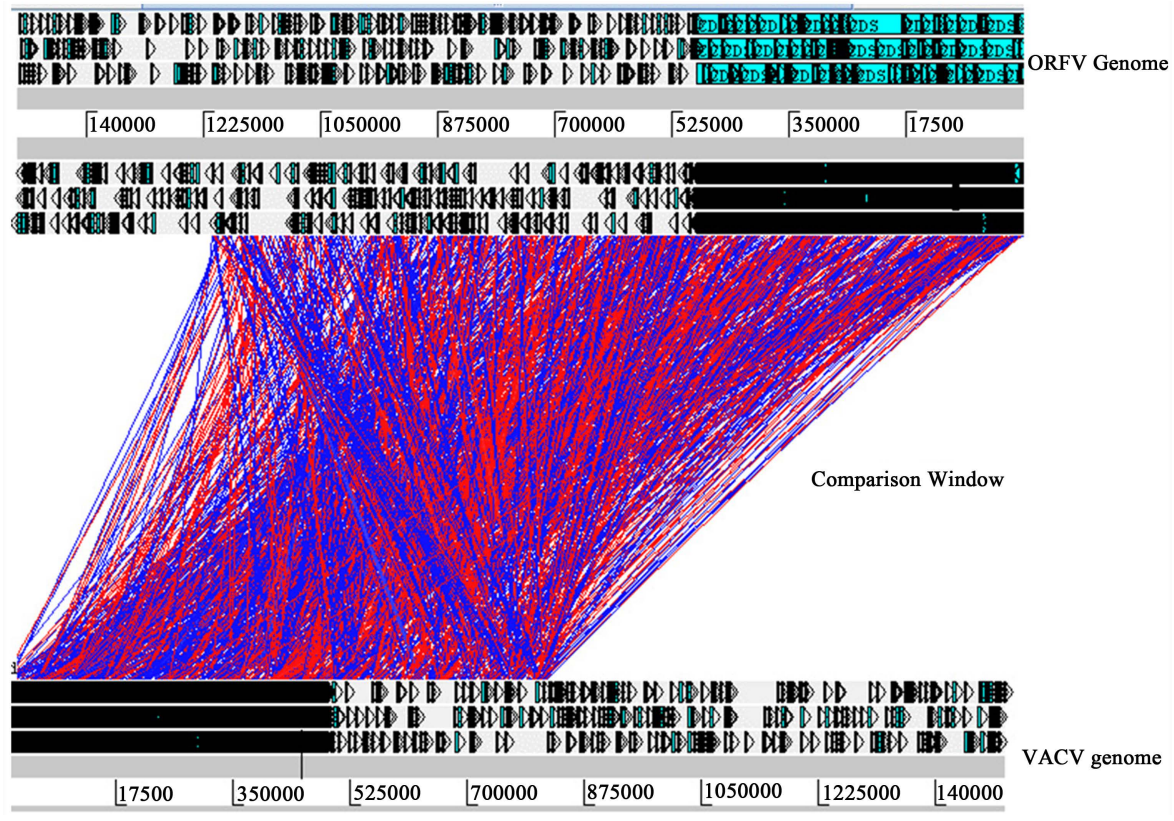
Figure 2. Signal peptide determination of ORF 039 by SignalP 3.0. Here, (a) and (b) represent the SignalP PAM matrix (PM) prediction and Hidden Markov Model (HMM) prediction, respectively.

3.5. Comparison of BPSV with ORFV

At the genomic level, BPSV and ORFV genomes share 67% to 75% nucleotide identity and contain 127 genes with the same relative order and orientation. Among them 15 genes are unique to PPVs. BPSV and ORFV contain 15 and 16 ORFs, respectively, that share no significant homology to known proteins while search for homology blast and are primarily located at the right end of the genome. Fourteen ORFs (001, 005, 012, 013, 024, 073, 113, 115, 116, 119, 120, 121, 124 and 125) was observed in both BPSV and ORFV. Besides, four ORFs (039, 116, 124 and 125) are present only in ORFV with 29% to 64% amino acid identities and one (ORF 133) is unique to BPSV. There are few ORFs which are distantly related with amino acid identity approximately up to 65%. Among thiese 30 distantly related ORF 10 are found as unique to 12 are unique to PPVs (ORFs 002, 005, 012, 013, 068, 113, 115, 116, 119, 120, 121 and 124). There are two ORFs 58, 57 that encode ankyrin repeat-containing proteins (ARPs) are observed only 50% identical between BPSV and ORFV. However, BPSV contains two (ORFs 003 and 004) additional ARPs in the left terminal genomic region which are not present in ORFV.



(a)



(b)

Figure 3. Comparative genomics of ORFV with other two closely related viruses BPSV and VACV. The red and blue bars indicate regions of similarity with red bars indicating corresponding regions that are oriented similarly and blue bars indicating regions oriented in opposite directions. Comparison of whole genome by ACT. The top view shows the subject sequence ORFV and the bottom view shows the query sequence: (a) Comparison of BPSV with ORFV; (b) Comparison of VACV with ORFV.

3.6. Comparison of VACV with ORFV

While compare with VACV genome with ORFV geneome, 13 ORFs (ORFs 061, 080, 088, 103, 109, 110, 112, 126, 128, 009, 016, 122 and 129) of ORFV show homology with VACV. Among these 13 homologue 10 genes have known function. Interestingly, ORFV lack homologues to VACV D9R. VACV D9R gene contains a *mutT* motif which is also present in VACV D10R that encodes a viral transcription protein and DNA ligase encoding gene VACV A50R. That suggest some other gene of ORFV which are not yet functionally characterized might have done the works for these genes. ORF 80 has homology with both VACV and BPSV. Besides, ORF 088, 109, 110 and 138 is orthologous with VACV.

3.7. Phylogenetic Analysis

In phylogenetic analysis based on the complete B2L gene, the ORF/09/Korea strain was closer to the Taiping isolate from Taiwan. ORF-ca1 and NZ-2-1 are closely related despite of different origin. Other ORF virus of sheep and Goat origin are less similar. Pseudocowpox and BPSV are distantly related with ORF virus (**Figure 4**).

4. Discussion

ORF virus shares specific genomic features with other poxviruses as VACV and BPSV, in terms of genome organization and gene content. Comparative genome sequences with other two closely related viruses VACV and BPSV here provide a comparative view of Parapoxvirus (PPV) genomics and basic knowledge of viral functions associated with virus replication and manipulation of cellular responses. Based on comparative genomic analysis, the genomes of BPSV and ORFV differ significantly which may be responsible for differences in host range. Modern genome analysis tools, such as Artemis Comparison Tools (ACT), promptus to understand the PPV biology which will permit the engineering of novel vaccine viruses and expression vectors with enhanced efficacy and greater versatility [30]. Nevertheless, we have identified four unique genes with potential importance as virulence factors and thus they could be vaccine candidates in the future. These genes should not correspond to horizontal gene transfer and their characteristic features may be consequences of the specific evolutionary cycle that shapes the ORFV gene repertoires in the context of their parasitic lifestyles [31]. ORF 039 with signal peptides and transmembrane domains may be directly toxic or confer association with the host. Therefore, further focus can be placed on subset of this for functional analysis. The function, subcellular location, average of

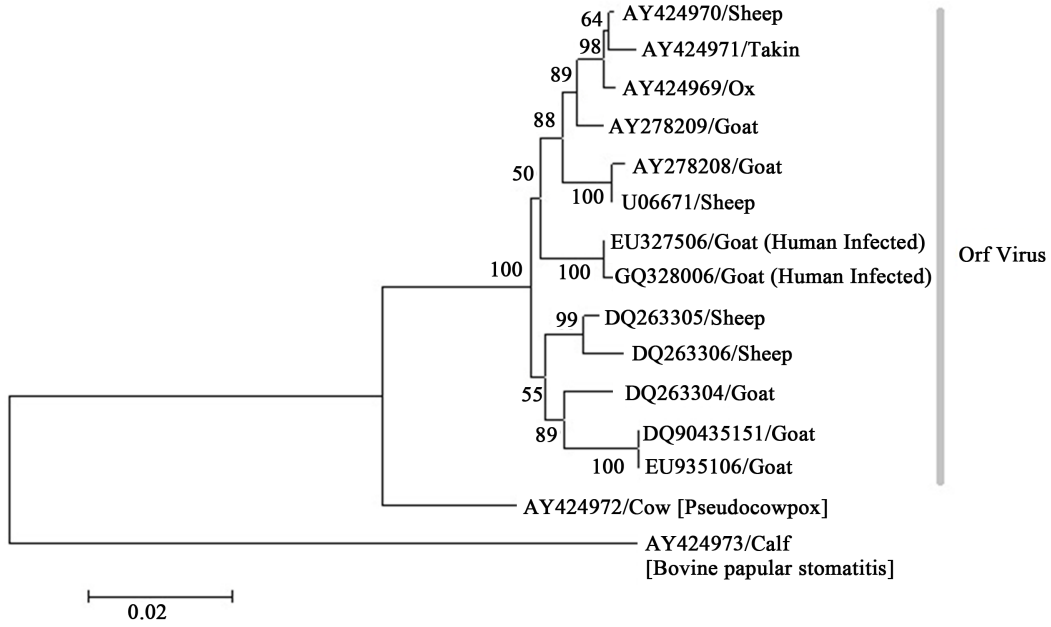


Figure 4. Phylogenetic analysis of different parapoxviruses. Phylogenetic tree is constructed based on viral B2L gene. Bootstrap values (derived from 1000 replicate neighbor-joining (NJ) trees estimated under the ML substitution model) are shown for key nodes > 50%.

hydrophobicity and protein regions that share a significant degree of sequence similarity with known protein family can be detected by using computational approach. Primarily 2D-PAGE might be used for membrane protein analysis for ORF 039 [32] [33]. Besides, Combination of nano liquid chromatography (NanoLC) and mass spectrometry (MS) can be used to detect the transmembrane domian of ORF 039 [33]. ORF 039 can be used for Insilco protein homology modeling. Identification of certain protein ligand of this protein may open a new door for the development of antibody for ORFV and also for potential drug target. Insilco drug target analysis may be carried out to find out the potentiality of the genes for further analysis.

Similar Insilco studies by the following different approach have been carried out to identify potential genes for therapeutic targets [34] [35]. Unique genes or proteins that are involved in a certain pathway or having certain characteristics like outer membrane protein, presence of unique protein ligand family, etc. are always an ideal candidate for drug target [35]-[37]. Insilco identification of target genes and prediction of drug candidate is a well-established methodology in drug discovery. Screening out the target genes and their corresponding drug by Insilco approach makes the drug discovery procedure more robust, quick and economically feasible [36].

The development of novel vaccine strain will control contagious pustular dermatitis in a small ruminant, potential source of leather, meat and milk which have a significant role in the economy of a country [13]. Therefore, control of such ORF related disease will have a significant role in economic development of a country. This study will be very much useful for further study of the evolution of the ORFV that may provide an encouragement for the development of new diagnostic tools and medicines.

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Supplementary

Table S1. Open reading frame predicted by GLIMMER.

ORF	Strand	Start	End	Gene Length
1	+	<1	192	192
2	+	1202	1522	321
3	-	2359	2943	585
4	-	3002	3271	270
5	-	3393	3581	189
6	-	3713	3928	216
7	-	4012	4491	480
8	-	4534	6138	1605
9	+	6158	6304	147
10	+	7141	7590	450
11	+	7744	9357	1614
12	+	9755	10,156	402
13	+	10,199	10,810	612
14	-	10,818	11,087	270
15	-	11,173	11,289	117
16	-	11,351	11,932	582
17	-	12,181	13,608	1428
18	-	13,621	13,923	303
19	+	13,950	14,552	603
20	-	14,935	15,918	984
21	-	16,463	17,434	972
22	+	17,569	18,045	477
23	+	18,064	18,540	477
24	+	18,811	19,338	528
25	+	19,305	20,030	726
26	-	19,997	20,701	705
27	-	20,798	21,610	813
28	-	21,757	22,551	795
29	-	22,590	22,739	150
30	+	22,730	23,482	753
31	-	23,605	25,140	1536
32	+	25,278	26,567	1290
33	-	27,075	28,196	1122
34	-	28,413	28,745	333
35	-	28,920	31,346	2427

Continued

36	+	31,355	31,474	120
37	+	32,233	33,021	789
38	-	33,410	33,604	195
39	+	34,418	34,579	162
40	-	34,743	35,006	264
41	-	35,558	35,794	237
42	+	35,779	36,792	1014
43	-	36,789	36,959	171
44	+	37,001	38,458	1458
45	-	38,442	38,660	219
46	-	38,706	38,927	222
47	+	38,944	39,954	1011
48	+	39,988	40,581	594
49	+	41,436	41,957	522
50	+	41,941	42,501	561
51	+	43,031	43,384	354
52	-	43,519	45,162	1644
53	+	45,168	45,356	189
54	-	45,362	45,868	507
55	+	45,859	46,137	279
56	-	46,783	47,007	225
57	-	47,176	47,814	639
58	-	47,818	47,949	132
59	-	48,233	48,556	324
60	+	48,538	49,251	714
61	-	49,773	50,096	324
62	-	50,112	50,375	264
63	-	50,393	50,863	471
64	-	51,131	52,129	999
65	-	52,173	52,604	432
66	-	53,208	54,563	1356
67	-	54,846	56,948	2103
68	+	57,117	57,950	834
69	-	58,236	58,499	264
70	-	58,908	59,063	156
71	+	59,091	59,192	102

Continued

72	+	59,189	61,795	2607
73	-	61,865	62,524	660
74	-	62,561	63,340	780
75	-	63,886	64,479	594
76	-	64,678	65,328	651
77	-	65,369	65,449	81
78	-	65,446	66,285	840
79	-	66,430	66,675	246
80	+	66,714	66,980	267
81	+	67,144	67,497	354
82	+	67,494	68,234	741
83	-	68,186	70,303	2118
84	+	70,285	70,617	333
85	-	70,961	71,335	375
86	+	71,419	73,173	1755
87	-	73,147	73,749	603
88	+	74,300	75,052	753
89	+	75,320	75,820	501
90	+	75,922	76,449	528
91	+	76,514	77,386	873
92	+	77,457	77,945	489
93	+	77,955	79,001	1047
94	-	78,978	79,379	402
95	-	79,466	80,149	684
96	+	80,407	81,369	963
97	+	81,448	81,885	438
98	+	82,006	82,302	297
99	+	82,688	83,254	567
100	-	83,884	85,164	1281
101	+	85,298	85,516	219
102	+	85,517	85,831	315
103	+	85,931	86,791	861
104	+	86,978	87,334	357
105	-	87,321	87,809	489
106	+	87,974	88,243	270
107	-	88,546	89,451	906

Continued

108	+	89,523	91,271	1749
109	+	91,231	91,446	216
110	+	91,510	91,839	330
111	+	91,972	92,316	345
112	-	92,311	92,628	318
113	-	92,695	92,910	216
114	-	93,010	93,402	393
115	-	93,399	93,608	210
116	+	94,084	95,055	972
117	+	95,363	95,500	138
118	+	95,787	96,410	624
119	+	96,498	97,526	1029
120	-	97,882	98,391	510
121	+	98,490	98,975	486
122	-	98,985	99,596	612
123	-	99,547	100,518	972
124	+	100,620	100,745	126
125	-	100,756	104,103	3348
126	-	104,386	105,942	1557
127	-	105,980	107,548	1569
128	-	107,740	108,495	756
129	-	108,790	109,185	396
130	-	109,283	109,429	147
131	+	109,430	110,494	1065
132	+	110,615	110,998	384
133	+	111,635	111,910	276
134	+	112,311	113,171	861
135	-	113,383	113,643	261
136	+	113,722	113,880	159
137	+	113,971	115,053	1083
138	-	115,099	115,314	216
139	+	115,578	116,282	705
140	+	116,462	116,947	486
141	-	117,511	117,828	318
142	-	118,195	118,887	693
143	+	118,939	119,223	285

Continued

144	-	119,220	119,606	387
145	+	120,087	120,728	642
146	-	121,035	121,802	768
147	+	121,856	123,634	1779
148	+	123,711	124,355	645
149	+	124,615	125,271	657
150	-	126,156	126,878	723
151	+	127,097	127,501	405
152	+	127,563	128,123	561
153	-	128,310	128,795	486
154	-	129,373	130,911	1539
155	-	131,656	133,017	1362
156	+	133,528	133,797	270
157	+	134,299	134,883	585
158	-	135,720	136,040	321
159	-	137,050	>137,241	192

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