

Case Report

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## Phylogenetic analysis and characterization of Korean orf virus from dairy goats: case report

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Published: 16 October 2009

Received: 4 July 2009

*Virology Journal* 2009, **6**:167 doi:10.1186/1743-422X-6-167

Accepted: 16 October 2009

This article is available from: <http://www.virologyj.com/content/6/1/167>

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### Abstract

An outbreak of orf virus infection in dairy goats in Korea was investigated. Suspected samples of the skin and lip of affected goats were sent to the laboratory for more exact diagnosis. Orf virus was detected by electron microscopy and viral DNA was identified by PCR. To reveal the genetic characteristics of the Korean strain (ORF/09/Korea), the sequences of the major envelope protein (B2L) and orf virus interferon resistance (VIR) genes were determined and then compared with published reference sequences. Phylogenetic analysis revealed that the ORF/09/Korea strain was closest to the isolates (Taiping) from Taiwan. This is believed to be the first report on the molecular characterization of orf virus in Korea.

### Background

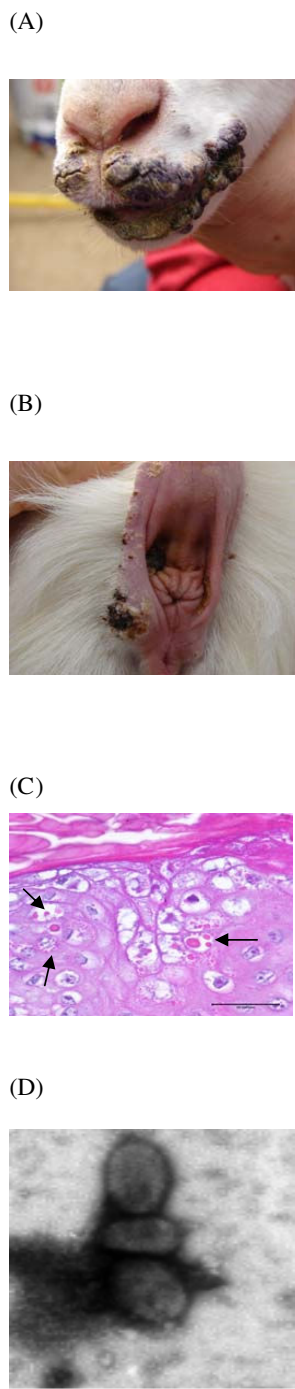
Contagious ecthyma (contagious pustular dermatitis; orf) is a common epitheliotrophic viral disease of sheep, goats, and wild ruminants and is characterized by the formation of papules, nodules, or vesicles that progress into thick crusts or heavy scabs on the lips, gingiva, and tongue. Orf virus is an oval, enveloped virus containing dsDNA genome within the genus *Parapoxvirus*, family *Poxviridae* [1]. The genus also includes pseudocowpox virus (PCPV) and bovine papular stomatitis virus (BPSV) in cattle and parapoxvirus of red deer in New Zealand. Zoonotic infection with orf virus is characterized by nodular and papillomatous lesions mainly on the hands, face, and mouth [2,3].

To reveal the genetic variation and characterization of parapoxvirus, the major virus envelope protein B2L and

virus interferon resistance (VIR) genes have been used recently [4-8]. Orf virus infection has been diagnosed occasionally in Korea since the outbreak of orf was reported clinically in the 1990s. Although a few studies have been conducted, molecular epidemiology based on gene sequences of orf virus has not been performed because the population of sheep and goats is low in Korea. In the present study, orf virus infection in dairy goats was identified by clinical diagnosis and PCR. The complete B2L and VIR genes were sequenced, and their phylogenetic trees were constructed.

### Case presentation

In April 2009, an exanthematic outbreak occurred in a farm with 400 dairy goats in the Chungbuk province. Sixty dairy goats presented with wart-like lesions on the lips, tongue, and around the anus (Fig. 1A and 1B). The clinical



**Figure 1**  
**Typical clinical cases of orf virus infection in dairy goats (A, B), histopathological findings (C) showing intracytoplasmic eosinophilic inclusion bodies (arrows) in the keratinocytes indicated virus-induced lesions (HE, bar = 35  $\mu$ m) and electron micrograph of the orf virus particle (D) showing typical oval-shaped morphology (bar = 100 nm).**

diagnosis was contagious ecthyma. Morbidity was 15% (60/400), and goats of all the ages ranging from 2 weeks to 1 month were affected. Dried scabs collected from affected goats were stored in a  $-70^{\circ}\text{C}$  freezer until the samples were used for further study.

The tissue samples were either fixed in 10% buffered formalin for histological examination, or were homogenized mechanically in PBS in a tube using a pellet pestle device. The homogenates were centrifuged at 3000 g for 5 min, and the supernatant was collected and negatively stained with 2% phosphotungstic acid for electron-microscopic examination of orf virus.

A PCR with OVS and OVA primers was performed as described previously [9]. In brief, the sequences of OVS and OVA primers are 5'-AGGCGGTGGAATGGAAAGA-3' and 5'-CCAGCAGGTATGCCAGGATG-3', respectively. The total viral DNA was extracted from scab samples using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The amplification were performed in a GeneAmp PCR System 2700 (Applied Biosystems) using the following conditions: a preliminary heating for 5 min at  $94^{\circ}\text{C}$ ; 10 cycles of 30 s at  $94^{\circ}\text{C}$ , 45 s at  $55^{\circ}\text{C}$ , and 1 min at  $72^{\circ}\text{C}$ ; 20cycles of 30 s at  $94^{\circ}\text{C}$ , 45 s at  $57^{\circ}\text{C}$ , and 1 min at  $72^{\circ}\text{C}$ ; and the final elongation of 5 min at  $72^{\circ}\text{C}$ . For phylogenetic analysis, a PCR was also performed using previously described primers [4,5]. The amplified gene was purified using an agarose gel DNA extraction kit (INtRON, Seongnam, Korea) and further cloned into pGEM-T vector (Promega, Madison, WI, USA) according to the manufacturer's instructions. Automated nucleotide sequencing of the VP60 gene insert was performed using an ABI 3130XL genetic analyzer with the BigDye<sup>®</sup> Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). The nucleotides at all positions were confirmed by three or more independent sequencing reactions in both directions. The published sequences of parapoxviruses were retrieved from GenBank for phylogenetic analyses (Table 1).

The B2L and VIR gene sequences of the orf virus, Korean strain were aligned with those of parapoxvirus sequences obtained from GenBank using Bioedit software (Ibis Biosciences, Carlsbad, CA, USA). A phylogenetic analysis was conducted using the Bioedit software and Molecular Evolutionary Genetics Analysis (MEGA) 3.1 software, with bootstrap values calculated from 1000 replicates [10]. The phylogenetic algorithm used for the building of the tree was the neighbor-joining method.

All clinical samples collected from the orf outbreak were positive using the previously described PCR method. The amplified PCR products were separated by electrophoresis and visualized by staining with ethidium bromide (EtBr).

**Table 1: Korean orf virus sequence and published parapoxvirus sequences used in the phylogenetic analysis**

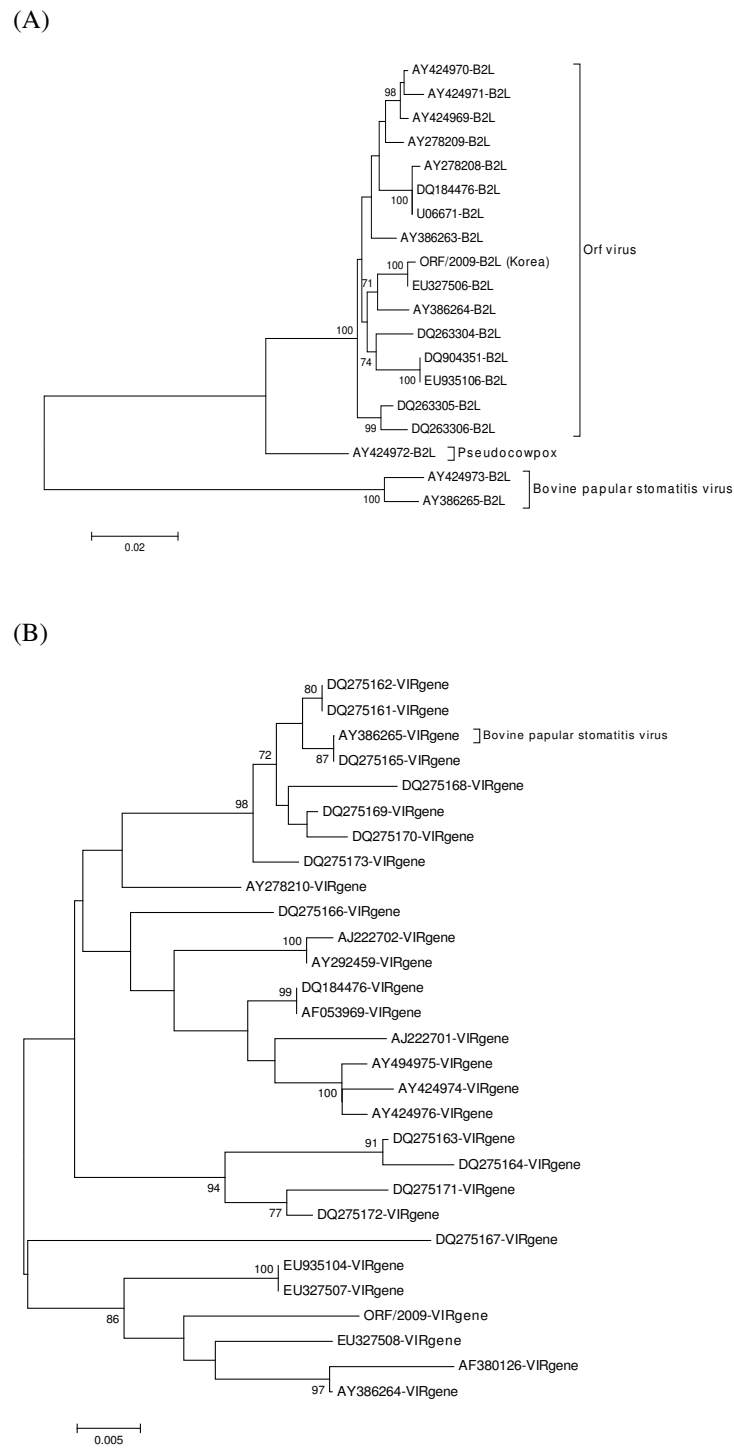
Published Sequences					
Description	Country of origin	Species of origin	Reference	Region of gene	Accession Number
ORF/2009-B2L	Korea	Goat	NA <sup>a</sup>	B2L	<a href="#">GQ328006</a>
ORF/2009-VIR	Korea	Goat	NA	VIR	<a href="#">GQ328007</a>
Orf-sh	USA	Sheep	Guo et al. (2004)	B2L	<a href="#">AY424970</a>
Orf-ta	USA	Takin	Guo et al. (2004)	B2L	<a href="#">AY424971</a>
Orf-mu	USA	Musk ox	Guo et al. (2004)	B2L	<a href="#">AY424969</a>
Orf-va1	USA	Goat	Guo et al. (2003)	B2L	<a href="#">AY278209</a>
Orf-ca1	USA	Goat	Guo et al. (2003)	B2L	<a href="#">AY278208</a>
Orf-ca2	USA	Sheep	Guo et al. (2003)	B2L, VIR	<a href="#">DQ184476</a>
NZ-2-1	New Zealand	Sheep	[13]Sullivan et al. (1994)	B2L	<a href="#">U06671</a>
OV-IA82	USA	Sheep	[14]Delhon et al. (2004)	B2L	<a href="#">AY386263</a>
Taiping	Taiwan	Goat	Chan et al. (2009)	B2L	<a href="#">EU327506</a>
OV-SA00	USA	Sheep	Delhon et al. (2004)	B2L, VIR	<a href="#">AY386264</a>
India 59/05	India	Goat	Hosamani et al. (2006)	B2L	<a href="#">DQ263304</a>
Nantou	Taiwan	Goat	Chan et al. (2007)	B2L	<a href="#">DQ904351</a>
Hoping	Taiwan	Goat	NA	B2L	<a href="#">EU935106</a>
India 67/04	India	Sheep	Hosamani et al. (2006)	B2L	<a href="#">DQ263305</a>
India 79/04	India	Sheep	Hosamani et al. (2006)	B2L	<a href="#">DQ263306</a>
PCPV-TQ	NA	Cow	Guo et al. (2004)	B2L	<a href="#">AY424972</a>
BPSV-RS	NA	Calf	Guo et al. (2004)	B2L	<a href="#">AY424973</a>
BPSV-BV	USA	Calf	Delhon et al. (2004)	B2L, VIR	<a href="#">AY386265</a>
1010/03 GR	Greece	Sheep	Kottaridi et al. (2006)	VIR	<a href="#">DQ275162</a>
30/96GR	Greece	Goat	Kottaridi et al. (2006)	VIR	<a href="#">DQ275161</a>
176/95GR	Greece	Sheep	Kottaridi et al. (2006)	VIR	<a href="#">DQ275165</a>
928/02GR	Greece	Sheep	Kottaridi et al. (2006)	VIR	<a href="#">DQ275168</a>
661/95IT	Italy	Sheep	Kottaridi et al. (2006)	VIR	<a href="#">DQ275169</a>
12129/00IT	Italy	Sheep	Kottaridi et al. (2006)	VIR	<a href="#">DQ275170</a>
6126/02IT	Italy	Sheep	Kottaridi et al. (2006)	VIR	<a href="#">DQ275173</a>
Orf-va2	USA	NA	Guo et al. (2004)	VIR	<a href="#">AY278210</a>
1710/03GR	Greece	Goat	Kottaridi et al. (2006)	VIR	<a href="#">DQ275166</a>
Orf-11-1	New Zealand	Sheep	McInnes et al. (1998)	VIR	<a href="#">AJ222702</a>
Orf-11-2	New Zealand	Sheep	NA	VIR	<a href="#">AY292459</a>
NZ-2-2	New Zealand	Sheep	[15]Mercer et al. (2006)	VIR	<a href="#">DQ184476</a>
NZ-2-2	New Zealand	Sheep	[16]McInnes et al. (1998)	VIR	<a href="#">AF053969</a>
MRI-Scab	New Zealand	Sheep	McInnes et al. (1998)	VIR	<a href="#">AJ222701</a>
Orf-ta	USA	Takin	Guo et al. (2004)	VIR	<a href="#">AY424976</a>
Orf-mu	USA	Ox musk	Guo et al. (2004)	VIR	<a href="#">AY424974</a>
Orf-sh	USA	Sheep	Guo et al. (2004)	VIR	<a href="#">AY424975</a>
155/95GR	Greece	Sheep	Kottaridi et al. (2006)	VIR	<a href="#">DQ275163</a>
759/01GR	Greece	Goat	Kottaridi et al. (2006)	VIR	<a href="#">DQ275164</a>
7389/03IT	Italy	Sheep	Kottaridi et al. (2006)	VIR	<a href="#">DQ275172</a>
13598/03IT	Italy	Sheep	Kottaridi et al. (2006)	VIR	<a href="#">DQ275171</a>
513/03GR	Greece	Goat	Kottaridi et al. (2006)	VIR	<a href="#">DQ275167</a>
Hoping	Taiwan	Goat	NA	VIR	<a href="#">EU935104</a>
Natou	Taiwan	Goat	[17]Chan et al. (2007)	VIR	<a href="#">EU327507</a>
Taiping	Taiwan	Goat	Chan et al. (2007)	VIR	<a href="#">EU327508</a>
GPDV	Taiwan	NA	Guo et al. (2003)	VIR	<a href="#">AF380126</a>

<sup>a</sup>NA indicates not available

The size of amplified PCR product was 708 bp (data not shown). Keratinocytes in the stratum spinosum showed vacuolation and ballooning degeneration. Intracytoplasmic eosinophilic inclusion bodies were found characteristically in keratinocytes (Fig 1C). Negatively stained orf virus particles were examined by electron microscopy, which revealed the oval-shaped morphology of *Parapoxvi-*

*rus*. The size of the observed virus particles was approximately 150-200 nm (Fig. 1D).

Additional PCR reactions were performed to obtain the full-length B2L and partial VIR genes. The amplified PCR products were 1206 bp for the full-length B2L gene and 552 bp for the partial VIR gene. The purified DNA was



**Figure 2**  
**Phylogenetic analysis of different parapoxviruses based on nucleotide sequences of B2L gene (A) and VIR gene (B).** The nucleotide sequences of diverse orf viruses were aligned using the Bioedit program and Mega 3 software. One thousand bootstrap replicates were subjected to nucleotide sequence distance and neighbor-joining methods, and the consensus phylogenetic tree is shown. All bootstrap values are displayed above the tree branches, and only bootstrap values >70% are shown.

sequenced for comparative analysis. The sequence comparison of the ORF/09/Korea strain demonstrated a high degree of identity among our Korean strain and other orf virus strains. In phylogenetic analysis based on the complete B2L gene, the ORF/09/Korea strain was closer to the Taiping isolate (EU327506) from Taiwan (Fig. 2A). The ORF/09/Korea strain and Taiping isolate showed 99.82% and 99.48% similarity at the nucleotide and amino acid levels, respectively. The deduced amino acid sequences of the complete B2L gene of the ORF/09/Korea strain and the other strains were aligned using Bioedit software. Unique amino acid substitutions were found at two positions (A/R<sup>250</sup>→D, I<sup>275</sup>→M).

In phylogenetic analysis based on the partial VIR gene, the ORF/09/Korea strain also was closer to the Taiping isolate (EU327508) from Taiwan (Fig. 2B). The ORF/09/Korea strain and Taiping isolate showed 97.8% and 97.6% similarity at the nucleotide and amino acid level, respectively. The deduced amino acid sequences of the partial VIR gene of the ORF/09/Korea strain and the other strains were aligned, and unique amino acid substitutions were found at two positions (N/D<sup>250</sup>→E, F<sup>275</sup>→S).

## Conclusion

Orf infection is endemic in Korea, and no vaccination has been implemented to control this disease. An increase in the dairy goat population in Korea has been observed over the past several years. Clinical diagnosis and electron microscopy have been used routinely to identify orf viruses in Korea. Recently, PCR methods have been used to detect the viruses [11,12]. The dairy goat is not a native species in Korea and has been imported from abroad. Several causes of orf outbreaks exist, and the phylogenetic analysis may indicate a hypothetical origin of the viral strains involved.

Although only one case from dairy goats that showed clinical signs was used in the present study, the ORF/09/Korea strain was closest to the isolates (Taiping) from Taiwan, according to phylogenetic analysis based on the B2L and VIR genes. However, it is difficult to conclude the precise route by which the Korean orf virus strain was introduced into Korea based on molecular analysis alone. Also, at present, all ruminants from Taiwan have been prohibited from importation into Korea because of the outbreak of foot-and-mouth disease in Taiwan. Therefore, more epidemiological data concerning the distribution of orf viruses in Korea and neighboring countries are needed. Our continuing work to characterize more isolates in Korea and neighboring countries should prove valuable in that regard.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

JKO\*, YHJ, and OSL participated in the planning of the project. KKL and HRK performed the PCR and phylogenetic analysis. ISR and KHL collected the samples and performed electron microscopy and histopathological examination. All authors read and approved the final manuscript.

## Acknowledgements

This study was supported by the National Veterinary Research and Quarantine Service, Ministry of Agriculture, Anyang, Republic of Korea.

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