

CASE REPORT

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Diagnosis and phylogenetic analysis of Orf virus from goats in China: a case report

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Abstract

Background: Orf virus (ORFV) is the etiological agent of contagious pustular dermatitis and is the prototype of the genus Parapoxvirus (PPV). It causes a severe exanthematous dermatitis that afflicts domestic and wild small ruminants.

Case presentation: In the present study, an outbreak of proliferative dermatitis in farmed goats. The presence of ORFV in tissue scrapings from the lips was confirmed by B2L gene polymerase chain reaction (PCR) amplification. The molecular characterization of the ORFV was performed using PCR amplification, DNA sequencing and phylogenetic analysis of the B2L gene.

Conclusion: The results of this investigation indicated that the outbreak was caused by infection with an ORFV that was closely related genetically to Nantou (DQ934351), which was isolated from the Tai wan province of China and Hoping (EU935106), which originated from South Korea in 2008. This is the first report of the phylogenetic analysis of ORFV from goats in China.

Background

The ORFV is the prototype member of the genus *Parapoxvirus*, which also includes *pseudocowpox virus* (PCPV) in cattle, *bovine papular stomatitis virus* (BPSV) in cattle, *squirrel parapoxvirus* (SPPV) and *parapoxvirus of red deer in New Zealand* (PVNZ) [1,2]. Contagious pustular dermatitis is a common viral skin disease that occurs in a range of species, not only in wild ruminants [3] but also in humans [4-6]. Humans with immunodeficiency diseases, in particular, can develop serious infections [7]. The diseases caused by ORFV have worldwide distribution and have been reported from many countries [1].

The disease not only has an economic impact on farmers worldwide but also has a considerable negative effect on animal welfare. Infected animals are sickly, fail to thrive, and are more susceptible to adventitious bacterial infections [8]. The typical progress of orf in goats and sheep moves from erythema, via vesicle formation, to pustules and then to scabs. Characteristic of the disease

are proliferative and often self-limiting lesions on the skin of the lips, on the oral mucosa and around the nostrils. Lesions can also be found occasionally on the teats of nursing animals and rarely on other organs[9]. Depending on the location of the lesions, animals may be unwilling to nurse, eat, or walk [10]. Primary lesions usually resolve spontaneously within 3-4 weeks [11]. The mortality rate related to orf is usually low, but it may be very high in small ruminants, especially when bacterial or fungal secondary infections occur [12,13]. The ORFV genome consists of linear double-stranded DNA (134-139 kb) [14]. The envelope gene (B2L) of the ORFV encodes for a highly immunogenic envelope protein of about 42 kDa [15]. A conventional PCR method that is based on the amplification of the B2L gene has been used for the detection of ORFV by PCR [1,16,17]. Molecular characterization and phylogenetic analysis [1,18,19] have been based on the complete sequence of the B2L gene.

In recent years, outbreaks of orf have occurred worldwide [1]. Although outbreaks of orf have occurred in China and have been confirmed, there are few reports available of the detailed molecular characteristics and phylogenetic analysis of the viruses involved. We report an outbreak of ORFV infection in goats from the Hubei province of the People's Republic of China in which the ORFV was verified by PCR of the full-length B2L gene.

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Comparative sequence analysis of the B2L gene from this outbreak of orf was carried out, and the phylogenetic relationship of the virus with other ORFV sequences available in GenBank was determined. This is the first report of the phylogenetic analysis of an ORFV in China in comparison with other isolates from other regions.

Case presentation

The outbreak reported in this study originated on a goat farm (114.52° E, 29.6° N) in the Hubei province of the People's Republic of China. On 23 March 2009, the farmer bought 655 goats from free-ranging herds and transported them a distance of about 800 km to the farm. On the third day, two goats presented with nodular lesions on the lips, tongue and around the mouth; there were 30 goats showed tubercular lesions three days later. To 31 July 2009, the incidence was approaching 60% and the mortality rate was 24.7% (162/655), although antiviral and antibiotic medicines were administered in the drinking water, by intramuscular injection or orally.

Two goats with typical clinical signs were selected and euthanized with an overdose of pentobarbital sodium. Complete post mortem examinations were performed and the macroscopic changes were recorded. Small samples of diseased tissue, about 6-8 mm³, were removed carefully from the nodular lesions using a sterile scalpel and stored in a sealed plastic tube with 50% glycerin Phosphate Buffered Saline(PBS) at 20°C until analysis. Primers designed for the amplification of the full length of the B2L gene [20,21] were synthesized (Takara dalian Co., Ltd). Tissue scrapings collected from the infected goats were triturated in 0.1 M PBS (1:10 V/V) and freeze-thawed twice between -20°C and 37°C, then placed at 4°C overnight. After centrifugation at 3000 rpm for 20 min at 4°C, DNA was isolated from the clarified supernatant using a Genomic DNA Purification Kit (Promega) and used as template in the PCR procedures. Tissue scrapings of healthy goat were treated with the same procedures and used as negative control.

Comparison of the B2L sequences with those available in the GenBank database was performed using the online BLAST programs. The sequence identities of nucleotides and amino acids were analyzed by the ClustalW method [22]. A phylogenetic tree based on the deduced amino acid sequences was constructed by the neighbor-joining method with 1000 bootstrap replicates using MEGA version 4.0 [23].

Discussion and Conclusion

The clinical signs seen in the goats included: multifocal to coalescing papillary, verrucose, proliferative, and ulcerated lesions in the epidermis of the muzzle and lips (Fig. 1). No visible lesions were found in other locations.

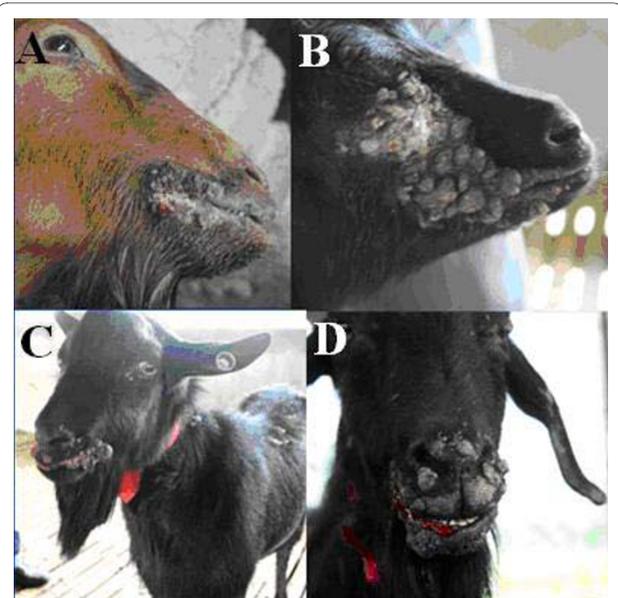
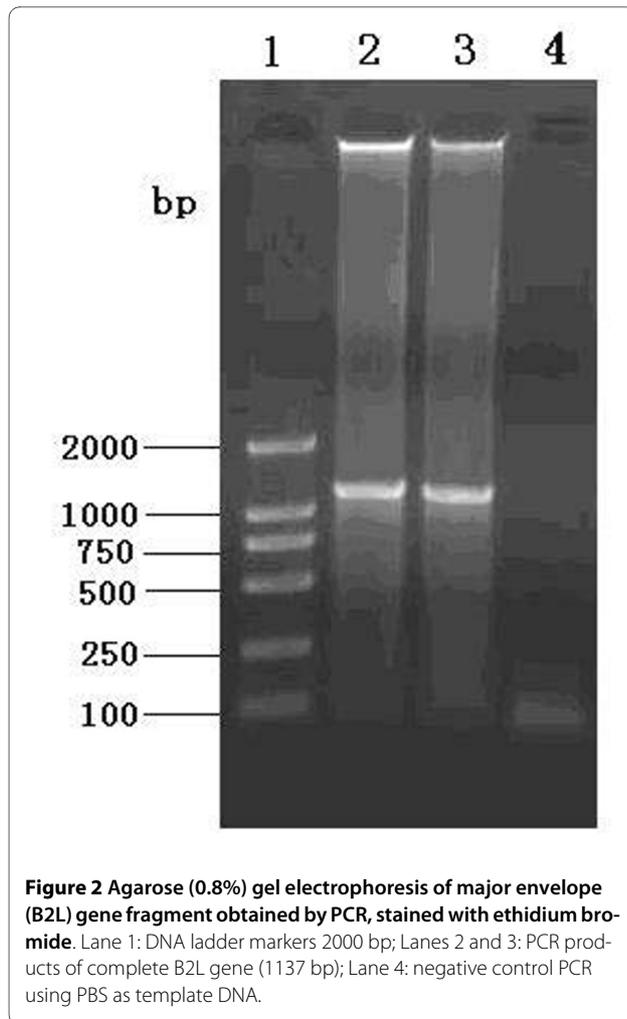


Figure 1 Representative clinical cases of ORFV infection in goats. (A) Goat with severe proliferative lesions of ecthyma around the lips. (B and C) Wart-like multiple nodules on the upper and lower eyelids. (D) Copious serosanguineous discharge appeared when the nodules burst.

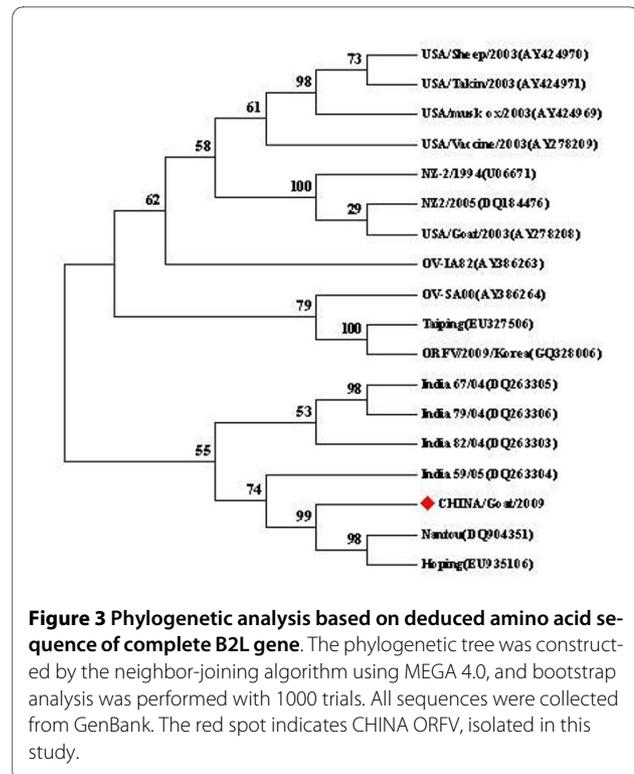
The orthodox methods of diagnosis that depend on pathologic examinations and clinical signs are inaccurate, virus isolation is thought to be a gold standard but it's time-consuming[14]. With the development of molecular biology, the PCR technique has become widely used to amplify the desired genomic fragments from tissue specimens, and it has become a powerful tool in molecular diagnosis. The PCR method is able to diagnose ORFV infection in field specimens from affected animals [2]. To confirm whether the causative agent was present in skin scrapings, PCR of the complete B2L gene was used in this study. The expected PCR fragments, approximately 1137 bp in length, were obtained from DNA which had been extracted from tissue scrapings; no fragments were obtained from the negative control (Fig. 2). To ensure the validity of the sequence as far as possible, DNA probest Taq enzyme was used in the PCR, and the PCR products were amplified directly from the skin scrapings. To gain further information about the virus, we sequenced the PCR product. The results showed that the B2L gene was 1137 bp in length and encoded 379 amino acids. The G+C ratio was 63.3%, which is consistent with the whole genome of the virus [1,24]. Sequences of the B2L gene of the virus were submitted to NCBI GenBank and assigned the accession number [GU320351](https://www.ncbi.nlm.nih.gov/nuclot/GU320351).

Using nucleotide sequences of the complete B2L gene stored in GenBank (Table 1), the phylogenetic relationships were explored using the neighbor-joining method and bootstrap analysis (Fig. 3). The results demonstrated



that the CHINA/Goat/2009 isolate obtained from this outbreak was closest to the Nantou (DQ934351) isolate obtained from the Tai wan province of China in 2006 and the Hopping (EU935106) isolate, which originated from South Korea in 2008 (Fig. 3). The percent identities and diversities of the deduced amino acid sequence of the B2L gene among the different strains of ORFV were calculated (Table 2) using the MegAlign function of DNASTAR software. The sequence analysis revealed high nucleotide and amino acid identity among the isolates from different countries; they shared 95.3%-99.7% sequence identity at the amino acid level. This is consistent with the fact that the central region of Parapoxviruses is generally conserved.

Orf is endemic in China, although a vaccination program has been performed to control the disease. Between 1980s and 1990s, orf occurred in eight provinces of China including Qinghai, Gansu, Tibet, Xinjiang, Liaoning, Jiangxi, Heilongjiang and Hebei. In recent years orf happened in the following provinces Innermongolia



(Jun.2005), Guangxi (Mar.2005), Shanxi (Apr.2005), Fujian (Aug.2005), Jilin (Mar.2006), Jiangsu(Nov.2006) and Beijing city (Apr.2006). More seriously, in 2005 there were seven women and four men were infected by ORFV in Fujian province. So, orf is a national zoonoses in China. In this study, we diagnosed an outbreak of orf in Chinese goats and determined its phylogenetic characteristics on the basis of the complete gene sequence of the major envelope protein (B2L). We conclude that the Chinese ORFV involved in this outbreak was closely related phylogenetically to Nantou (DQ934351) and Hopping (EU935106). This is the first report to provide phylogenetic information about an ORFV strain in China, which will be of use for prospective studies in public health.

List of abbreviations

ORFV: Orf virus; PCPV: pseudocowpox virus; BPSV: bovine papular stomatitis virus; SPPV: squirrel parapoxvirus; PVNZ: parapoxvirus of red deer in New Zealand; PCR: polymerase chain reaction; PBS: phosphate buffered solution; bp: base pair; kb: kilobase.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

XL was the leader of the project. KZ carried out most of the studies and drafted the manuscript. JH and JY amplified the complete B2L gene. HZ, ZL and YS provided consultation and preparation of the final report. All authors read and approved the final manuscript.

Table 1: Detailed information on the ORFV used in the analysis; "---" indicates host species unknown.

S.No.	Virus strains	Country and year of isolation	Accession Number	Host species
1	NZ2/2005	New Zealand 2005	DQ184476	---
2	USA/Goat/2003	USA 2003	AY278208	Goat
3	USA/Vaccine/2003	USA 2003	AY278209	---
4	OV-IA82	USA 1982	AY386263	Lamb
5	OV-SA00	USA 2003	AY386264	Kid
6	USA/musk ox/2003	USA 2003	AY424969	Musk ox
7	USA/Sheep/2003	USA 2003	AY424970	Sheep
8	USA/Takin/2003	USA 2003	AY424971	Takin
9	India 82/04	India 2004	DQ263303	Goat
10	India 59/05	India 2005	DQ263304	Goat
11	India 67/04	India 2004	DQ263305	Sheep
12	India 79/04	India 2004	DQ263306	Sheep
13	Nantou	CHINA tw 2006	DQ904351	Goat
14	Taiping	CHINA tw 2007	EU327506	---
15	Hoping	South Korea 2008	EU935106	Goat
16	ORFV/2009/Korea	South Korea 2009	GQ328006	Dairy goat
17	NZ-2/1994	New Zealand 1994	U06671	Sheep
18	CHINA/Goat/2009	China	GU320351	Goat

Table 2: The percentage identities and diversities of deduced amino acid sequences of the B2L gene among ORFV strains.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Virus strains
1		99.5	98.7	97.6	96.3	97.4	99.5	97.1	97.1	97.6	97.6	98.4	98.2	96.8	97.1	97.4	96.6	96.8	1 CHINA Goat 2009
2	0.3		98.9	97.9	96.6	97.6	99.7	97.4	97.4	97.9	97.9	98.7	98.4	97.1	97.4	97.6	96.8	97.1	2 Hoping(EU935106)
3	1.1	0.8		98.2	96.8	97.4	98.9	97.6	97.6	97.6	98.2	98.4	98.2	97.4	97.6	97.9	97.1	97.4	3 India 59 05(DQ263304)
4	2.1	1.9	1.6		98.4	98.4	97.9	98.7	98.7	97.1	97.6	97.9	97.6	98.4	97.1	97.4	96.6	97.4	4 India 67 04(DQ263305)
5	3.5	3.2	3.0	1.3		97.1	96.6	97.4	97.4	95.8	96.3	96.6	96.3	97.1	95.8	96.0	95.3	96.0	5 India 79 04(DQ263306)
6	2.4	2.1	2.4	1.3	2.7		97.6	97.4	97.4	96.8	97.9	97.6	97.4	97.1	96.8	97.1	96.3	97.1	6 India 82 04(DQ263303)
7	0.3	0.0	0.8	1.9	3.2	2.1		97.4	97.4	97.9	97.9	98.7	98.4	97.1	97.4	97.6	96.8	97.1	7 Nantou (DQ904351)
8	2.7	2.4	2.1	1.1	2.4	2.4	2.4		99.7	97.1	98.2	97.9	97.6	99.5	98.2	98.4	97.6	98.4	8 NZ-2 1994(U06671)
9	2.7	2.4	2.1	1.1	2.4	2.4	2.4	0.0		97.1	98.2	97.9	97.6	99.5	98.2	98.4	97.6	98.4	9 NZ2 2005(DQ184476)
10	2.1	1.9	2.1	2.7	4.1	3.0	1.9	2.7	2.7		97.1	97.9	99.2	96.8	96.6	96.8	96.0	96.3	10 ORFV 2009Korea(GQ328006)
11	2.1	1.9	1.6	2.1	3.5	1.9	1.9	1.6	1.6	2.7		97.4	97.6	97.9	98.7	98.9	98.2	98.4	11 OV-IA82(AY386263)
12	1.6	1.3	1.6	2.1	3.5	2.4	1.3	2.1	2.1	2.1	2.7		98.4	97.6	97.4	97.6	96.8	97.1	12 OV-SA00(AY386264)
13	1.6	1.3	1.6	2.1	3.5	2.4	1.3	2.1	2.1	0.5	2.1	1.6		97.4	97.1	97.4	96.6	96.8	13 Taiping (EU327506)
14	3.0	2.7	2.4	1.3	2.7	2.7	2.7	0.3	0.3	3.0	1.9	2.4	2.4		97.9	98.2	97.4	98.2	14 USA Goat 2003(AY278208)
15	2.7	2.4	2.1	2.7	4.1	3.0	2.4	1.6	1.6	3.2	1.1	2.7	2.7	1.9		98.9	98.2	98.4	15 USA musk ox 2003(AY424969)
16	2.4	2.1	1.9	2.4	3.8	2.7	2.1	1.3	1.3	3.0	0.8	2.4	2.4	1.6	0.8		98.9	98.9	16 USA Sheep 2003(AY424970)
17	3.2	3.0	2.7	3.2	4.6	3.5	3.0	2.1	2.1	3.8	1.6	3.2	3.2	2.4	1.6	0.8		98.2	17 USA Takin 2003(AY424971)
18	3.0	2.7	2.4	2.4	3.8	2.7	2.7	1.3	1.3	3.5	1.3	3.0	3.0	1.6	1.3	0.8	1.6		18 USA Vaccine 2003(AY278209)

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