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Identification and phylogenetic analysis of an orf virus strain isolated in Anhui Province, East-central China, in 2018

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Summary. – Orf, also called contagious ecthyma or contagious pustular dermatitis, is a significant zoonotic disease that primarily affects goat and sheep globally. Currently, the infection by orf virus (ORFV) has been observed in different host species worldwide, including China. Here, a suspected outbreak of orf infection in a goat farm in Anhui Province in 2018 was investigated. Through PCR, electron microscopy, and cell culture techniques, we confirmed that the outbreak was caused by ORFV. Consequently, the orf virus strain was named the AH/LA/2018 strain. The amplified and sequenced ORFV011 (B2L) and ORFV059 (F1L) genes were used to construct phylogenetic trees to elucidate the genetic characteristics of the ORFV and the molecular epidemiology of orf. The present study is the first systematic evolution analysis of the ORFV strain isolated in Anhui Province. The results of this study will be helpful to better understand the characteristics of ORFV, to help prevent and control the transmission of ORFV at an early stage in China.

Keywords: Anhui Province; goat; orf virus; phylogenetic analysis

Introduction

Orf virus (ORFV), the etiological agent of contagious pustular dermatitis or scabby mouth, commonly infects goat and sheep flocks worldwide. It is a typical member of the genus *Parapoxvirus* (the subfamily *Chordopoxvirinae*, the family *Poxviridae*) that includes bovine papular stomatitis virus (BPSV), pseudocowpox virus (PCPV), squirrel parapoxvirus (SPPV), and parapoxviruses of red deer in New Zealand (PVNZ) (Fleming *et al*., 2015; Spyrou and Valiakos, 2015). Orf primarily affects goats, sheep, and other ruminants, including reindeer, camels, and musk oxen, globally (Khalafalla *et al*., 2015; Kummeneje and Krogsrud, 1979; Schmidt *et al*., 2013; Vikoren *et al*., 2008). Further, the ORFV can also be transmitted to humans, causing an occupational hazard in butchers, goat and sheep shearers, farmers, and veterinary surgeons, predominantly damaging the skin of the hands (Al-Qattan, 2011; Georgiades *et al*., 2005). This virus can infect goat and sheep of all ages, causing significantly high morbidity in lambs (reaching up to 100%); however, the associated mortality is low, although reinfection lesions can be fatal. The clinical manifestation of the disease is mainly characterized by self-limiting maculopapular, inflammatory, and proliferative scabby lesions in the skin around the lips, muzzle, nostrils, and udder. The genome of ORFV is a linear double-stranded DNA approximately 138 kb long, with high G + C content, comprising central conserved regions (ORFs009-111) and two identical inverted terminal repeat regions (ORFs001-008 and ORFs112-134). The virus particle, which appears like a ball of wool, contains 130–134 putative genes that encode different proteins (Fleming *et al*., 2015). The ORFV011 (B2L) and ORFV059 (F1L) genes, located in the conserved region, can stimulate the body to produce a

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Abbreviations: BPSV = bovine papular stomatitis virus; CPE = cytopathic effect; OFTu = ovine fetal turbinate; ORFV = orf virus; PCPV = pseudocowpox virus

robust immune response (Friebe *et al*., 2011; Sullivan *et al*., 1994). During recent years, ORFV011 (B2L) and ORFV059 (F1L) genes have been used as markers in studies on genetic characteristic and molecular epidemiology (Oem *et al*., 2013).

During the past few years, cases of orf infection have been reported in several countries (Abdullah *et al*., 2015; Bora *et al*., 2012; Gelaye *et al*., 2016; Peralta *et al*., 2015). Frequent outbreaks of orf have been reported in Northwest, Northeast, East, Southern, and Central China (Chen *et al*., 2017; Duan *et al*., 2015; Li *et al*., 2012, 2013; Zhang *et al*., 2016). The disease not only causes enormous economic losses to the sheep and goat breeding industry, but also threatens human health worldwide (Spyrou and Valiakos, 2015). Hence, it is necessary to obtain more epidemiological information about this disease to monitor and control the occurrence of orf effectively. In this article, we report the outbreak of orf infection in a goat farm located in Lu'an city, Anhui province, in the east-central districts of China, in 2018. We used PCR, electron microscopy, and cell culture techniques to confirm and diagnose the outbreak. Moreover, to further prevent and control the disease, it is essential to know the evolutionary origin of ORFV. We amplified and sequenced the ORFV011 (B2L) and ORFV059 (F1L) genes and constructed phylogenetic trees to elucidate the relationship between the isolated strain and other ORFV strains. To some extent, the present study provides not only the evolutionary information of ORFV isolated from the east-central regions of China, but also reports epidemic data of orf, which is prevalent in China. The information in this article can help prevent and control the disease.

Materials and Methods

Clinical samples and outbreak data. On January 4, 2018, three male goats in a farm were suspected to be infected with ORFV. The farm is located in Lu'an City (31°43'45.76"N, 115°55'44.70"E), Anhui Province, China. The clinical symptoms of the disease, mainly scabbed lips and nose, severely affected the feeding of animals. The symptoms in the breast and abdomen were mild. Therefore, we collected lip scabs and stored them in the laboratory in a -80°C refrigerator until further analysis.

Preparation of the primary OFTu cell culture. After anesthetizing a pregnant goat, the abdominal surface was depilated and disinfected with 75% alcohol for 10 min. The uterus was aseptically dissected, the embryo was removed, and the turbinates from goat embryos were separated and washed thrice with phosphate-buffered saline (pH 7.2). The turbinates were then cut into small pieces in serum-free Dulbecco's modified Eagle medium (DMEM, Hyclone, USA) and digested with 12.5 g/l trypsin in a 37°C water bath for 20 min. The digested sample was centrifuged at 1,500 × *g* for 10 min. The cells were harvested and resuspended in DMEM supplemented with 10% fetal bovine serum. The collected cell suspension was counted, diluted to a concentration of 5×10^5 cells/ml, inoculated in a 6-well plate, and placed in a 5% CO_2 incubator at 37°C. All animal experiments were approved by the Committee on the Animal Ethics of Anhui Agricultural University. Experiments were carried out in accordance with the approved guidelines (No. AHAU-2018-034).

Virus isolation and morphological identification. The scab samples were mechanically triturated in phosphate-buffered saline (PBS) containing 1% antibiotics (100 mg/ml streptomycin and 100 mg/ml penicillin), freeze-thawed thrice at -80°C, placed in a microcentrifuge tube, and centrifuged at 6,000 × *g* for 20 min. After centrifugation, the supernatant was filtered through a 0.22 μm filter, and the monolayer of ovine fetal turbinate (OFTu) cells was incubated for 2 h at 37°C. The cells were washed thrice with phosphate-buffered saline, cultured in DMEM supplemented with 2% fetal bovine serum, and cytopathic effect (CPE) was observed. When approximately 80%–90% of the cells exhibited CPE, repeated freezing and thawing was performed, and then the cell debris was removed by centrifugation at 1,500 × *g* for 5 min at 37°C. The supernatant containing mature viral particles was stained with 2% phosphotungstic acid and observed using an electron microscope (HT7700, Hitachi High-Technologies Corporation, Japan).

DNA extraction and gene amplification. The viral DNA was directly extracted from the collected suspensions using the DNA mini kit (Tiangen, China) according to the protocol of the manufacturer. The extracted genomic DNA was stored in sterile Eppendorf tubes at -20°C for the PCR. To amplify the entire fragments of ORFV011 (B2L) and ORFV059 (F1L) genes, two primer pairs were designed and synthesized using the complete genomic sequence of Orf virus strain GO (Acc. No. KP010354) isolated from goats as a template in GenBank. The primer pairs were synthesized by Sangon Biotech (China): B2L-Up, 5'-ATGTGGCCGTTCTCCTCCATCC-3'; B2L-Down: 5'-TTAATTTATTGGTTTGCAGAACT-3'; F1L-Up: 5'-TCACACGATGGCCGTGACCAGCAGC-3'; and F1L-Down: 5'-ATGGATCCACCCGAAATCACGGCCT-3'. The genes were amplified by the PCR in a 50 μl reaction mixture. The PCR amplification conditions for ORFV011 (B2L) gene was as follows: initial pre-denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 62°C for 30 s, extension at 72°C for 70 s, and final extension cycle at 72°C for 10 min; and for ORFV059 (F1L): 30 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 70 s, final extension for 10 min at 72°C. The PCR products were separated by 1% agarose gel electrophoresis with Gold View I (Solarbio, China) for 30 min at 100 V, purified using the Gel extraction kit (Tiangen), ligated into the pMD19-T vector, and sequenced in both orientations. The sequences were assembled and edited using DNAMAN software (Lynnon BioSoft, Canada).

Phylogenetic analysis. The nucleotide (nt) and amino acid (aa) sequence identities of the isolated strain were aligned with the previously published corresponding sequences of parapoxvirus reference strains (ORFV strains (n = 38), BPSV strains (n = 4),

Table 1. Reference strains used for phylogenetic analysis

Reference strains	Host species	Origin	Genes	GenBank Acc. No.
AH/LA/18	*Goat*	*China*	*011/059*	*In our study*
YX	Goat	China	011/059	KP010353
GO	Goat	China	011/059	KP010354
NP	Goat	China	011/059	KP010355
SJ1	Goat	China	011/059	KP010356
NA1/11	Sheep	China	011/059	KF234407
FJ-SJ2	Goat	China	011/059	KC568397/KC568408
SDLC	Goat	China	011/059	KP339952/KP339950
SDTA	Goat	China	011/059	KP339951/KP339949
FJ-ZX	Goat	China	011/059	KC568400/KC568411
FJ-DS	Goat	China	011/059	KC568390/KC568401
FJ-FQ	Goat	China	011/059	KC568391/KC568402
FJ-GT	Goat	China	011/059	KC568394/KC568405
GDQY	Goat	China	011 /059	KM583893/KM583894
FJ-LJ2015	Goat	China	011/059	KU199828/KU199838
Guizhou	Goat	China	011/059	KP994595/KP057582
NZ2	Sheep	New Zealand	011/059	DQ184476
D1701	Sheep	Germany	011/059	HM133903
OV-IA82	Sheep	USA	011/059	AY386263
OV-SA00	Goat	USA	011/059	AY386264
Nantou	Goat	Taiwan	011	DQ904351
Taiping	Goat	Taiwan	011	EU327506
FJ-YT2015	Goat	China	011	KU199832
FJ-FZ2014	Goat	China	011	KU199824
Gansu	Goat	China	011	KC485343
ShanXi/2011	Goat	China	011	JN565696
Shanxi	Goat	China	011	HQ202153
HuB/XN	Goat	China	011	JQ904786
LiaoNing/2010	Goat	China	011	HQ694773
India59/05	Goat	India	011	DQ263304
ORF/2010/B2L-1	Goat	Korea	011	JX968988
ORFV/2009/Korea	Goat	Korea	011	GQ328006
NE2	Goat	Brazil	011	JN088051
ATARC/O01/2010	Sheep	Ethiopia	011	KT438522
FJ-LY2014	Goat	China	059	KU199839
FJ-LJ2014	Goat	China	059	KU199837
Xingjiang	Goat	China	059	KF703748
YM	Goat	China	059	KJ610842
Jilin-Nongan	Sheep	China	059	JQ271535
PCPV F00.120R	Reindeer	Finland	011	GQ329669
PCPV VR634	Reindeer	New Zealand	011	GQ329670
PCPV F07.798R	Reindeer	Finland	011	JF773692
BPSV AR02	Calf	USA	011/ 059	AY386265
BPSV BV-TX09c5	Bos taurus	USA	011/ 059	KM875471
BPSV BV-TX09c15	Bos taurus	USA	011/ 059	KM875470
BPSV BV-TX09c1	Bos taurus	USA	011/ 059	KM875472

PCPV strains (n = 3)) obtained from GenBank (Table 1), by the Clustal W method using the MegAlign program in the DNASTAR software package (USA). The phylogenetic trees were constructed based on the Kimura-2 parameter model and by the neighbor-joining method with 1,000 bootstrap replicates using the MEGA 6.0 software to analyze evolutionary relationships.

Fig. 1

Clinical symptoms of orf
Classic clinical lesions on the skin around the mouth and muzzle of orf virus-infected goats.

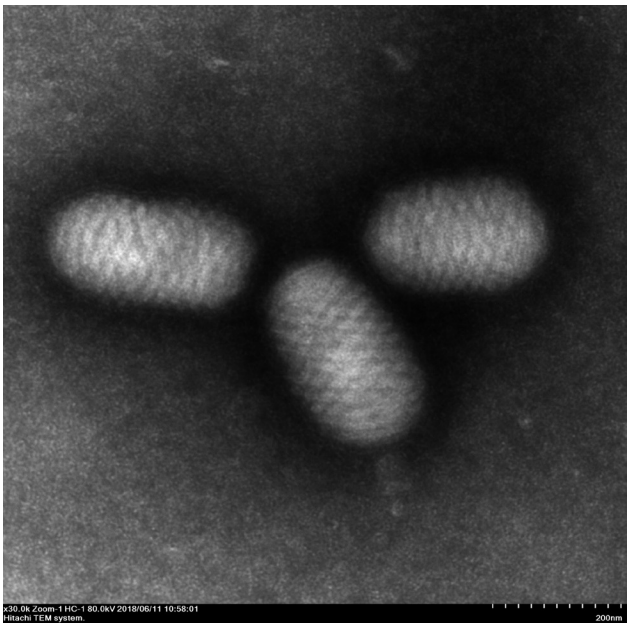

Fig. 3

Electron microscopic examination of the ORFV strain AH/LA/18 from infected OFTu cells
We observed viral particles of length ~260 nm and width ~180 nm (Scale bar = 200 nm).

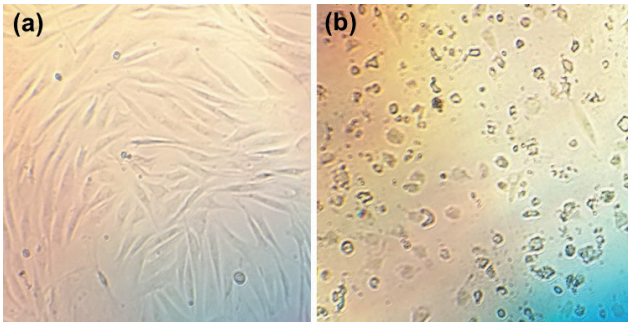

Fig. 2

Cytopathic effect of the primary ovine fetal turbinate cells infected with ORFV under a light microscope
Uninfected ovine fetal turbinate (OFTu) cells and OFTu cells 5 days after the inoculation with ORFV AH/LA/18.

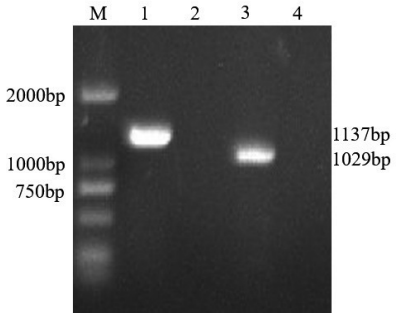

Fig. 4

Agarose gel electrophoresis of ORFV011 (B2L) and ORFV059 (F1L) genes obtained by PCR amplification
Agarose (1%) gel electrophoresis was stained with Gold View I. M: 2000-bp DNA ladder; Lane 1: PCR products of ORFV011 (B2L) gene of the ORFV AH/LA/18 strain with size of 1137bp ; Lane 3: PCR products of ORFV059 (F1L) of the ORFV AH/LA/18 strain with size of 1029bp; Lane 2, 4: extracted genomic DNA from uninfected OFTu cells (negative control).

Results

Orf typical clinical symptoms

The infected goats exhibited typical clinical symptoms of ORFV, characterized mainly by lip ulceration and crusting, papules around the nostrils, and blisters in the oral mucosa (Fig. 1). The scabs were collected for virus identification, PCR amplification, and phylogenetic tree analysis.

OFTu cells infection with ORFV AH/LA/18 strain

OFTu cells are often used for the separation of ORFV. In accordance with the method mentioned above, the healthy OFTu cells were inoculated with ORFV AH/LA/18 strain suspension and cultured in 1×DMEM.

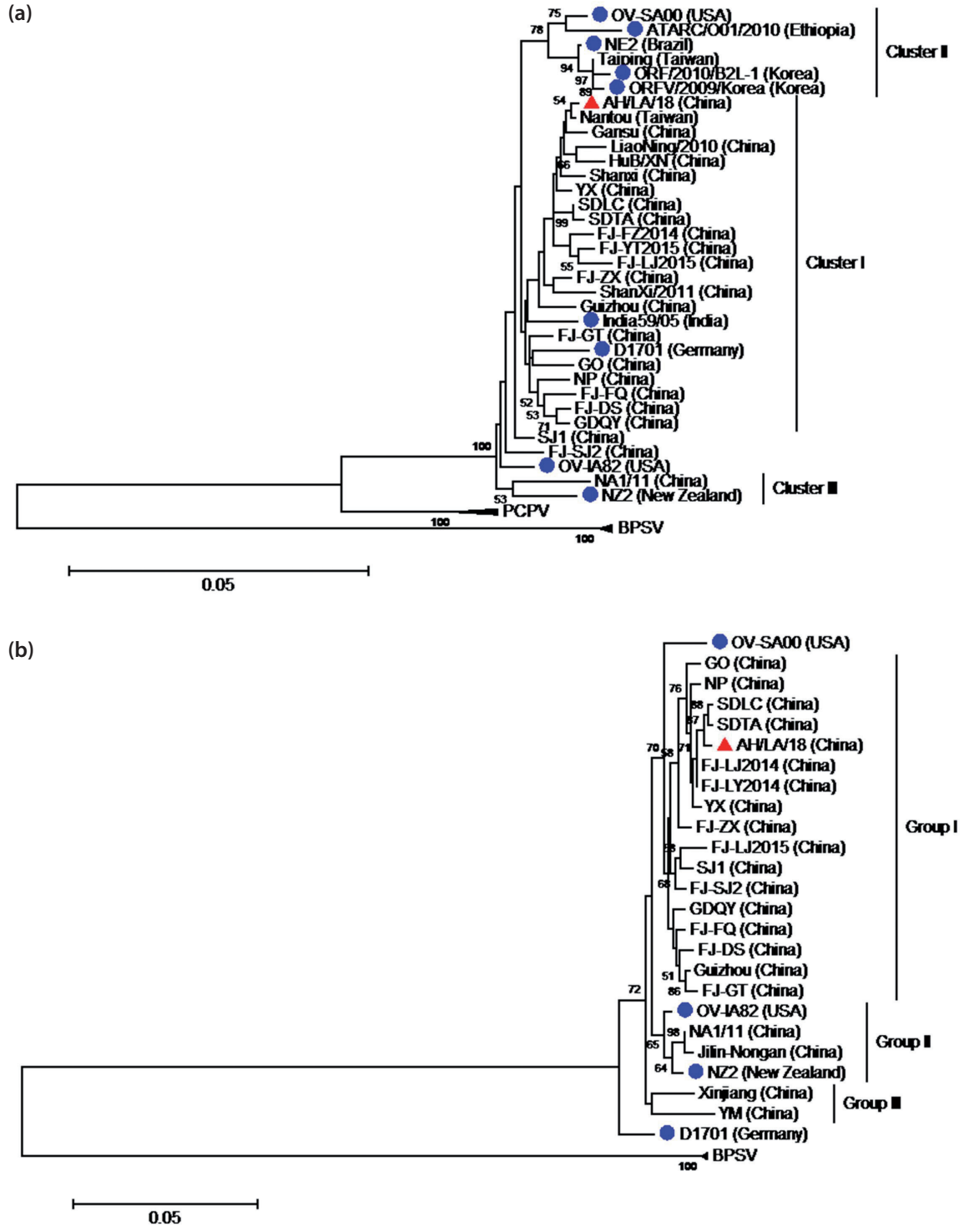

Fig. 5

Phylogenetic analysis of ORFV

Phylogenetic analysis of ORFV011 (B2L) **(a)** and ORFV059 (F1L) **(b)** genes of the isolated ORFV strain with the corresponding gene of different parapoxviruses. Red triangles represent the strain isolated in the present study, and blue circular dots represent the strains isolated from other countries except China. The alignment of a nucleotide sequence by the Clustal W method. The MEGA 6.0 software based on the Kimura-2 parameter model was used to construct the phylogenetic tree and evaluate the evolutionary relationship among different strains. A neighbor-joining algorithm with 1,000 bootstrap replicates was used. The bootstrap values (%) of ≥50% are displayed at tree branch node. The scale bar demonstrates branch length.

After 5 days, we observed that OFTu cells swelled, detached, and became round (Fig. 2b). This phenomenon was not observed in the uninfected group (Fig. 2a).

Morphological identification

For further identification we used electron microscopy. We have examined the supernatant of OFTu cells infected with ORFV AH/LA/18 strain. The viral particles of length ~260 nm and width ~180 nm were detected. The viral particle presented the typical elliptical pile-like structure with a helical cross pattern; it appeared rope-like in cross sections (Fig. 3).

PCR amplification

Using the primers previously designed and synthesized, the extracted DNA was used as a template for amplification by PCR. The uninfected group was used as a negative control. The electrophoresis of the amplified products presented bands of size 1137 and 1029 bp. There was no band present in the negative control (Fig. 4). The sequence of the ORFV011 (B2L) and ORFV059 (F1L) genes was edited and deposited in GenBank. Acc. Nos. ORFV011 (B2L), MH516005; ORFV059 (F1L), MH516004.

Phylogenetic analysis

Sequence alignments of nt and aa sequences based on ORFV011 (B2L) showed a high degree of identity with other ORFV strains in GenBank (Table 1). The nt and aa identities of AH/LA/18 strain with other ORFV strains were 97.3%–99.8% and 97.4%–99.5%, respectively. Among them, the ORFV011 (B2L) gene sequence of the ORFV AH/LA/18 strain presented a 99.8% nt sequence identity with the sequence of virus isolated from Nantou (data not shown). The phylogenetic trees based on the ORFV011 (B2L) gene, including 34 ORFV, 3 PCPV, and 4 BPSV strains, indicated that they form three independent clades (ORFV, PCPV, and BPSV). The PCPV strains are closer to the ORFV strains than the BPSV strains. The ORFV clade was mainly divided into three clusters. The AH/LA/18 strain belonged to Cluster I (23 strains), which is composed of isolates from China (21/23, 91.3%) and other countries (2/23, 8.7%). In addition, Cluster II (OV-SA00, ATARC/O01/2010, NE2, Taiping, ORF/2010/B2L-1, and ORFV/2009/Korea) and Cluster III (NA1/11 and NZ2) contained only two strains from China. The AH/LA/18 strain shared high homology with the strain from Nantou (Fig. 5a). The nt and aa sequence analysis of ORFV059 (F1L) gene revealed that the isolates from Anhui Province shared 96.3%–99.6% and 95.6%–100% identities with that of the other ORFV strains from the different districts, respectively (Table 1). The phylogenetic tree of ORFV059 (F1L) showed that the ORFV clade is also divided into three groups. The isolates clustered together with SDLC and SDTA in Group I which contained 18 strains from the mainland of China (18/18, 100%) (Fig. 5b).

Discussion

Orf is a ubiquitous zoonotic and transboundary disease, which is prevalent in goat and sheep breeding regions. It is also an endemic severe disease in China and has been classified as a class I animal disease (Li *et al*., 2013). In recent years, there have been reports of outbreaks of sheep aphthous diseases in developing countries, including in mainland China that have caused dramatic economic losses. Some researchers have analyzed the possible reasons and a few vaccine projects have been implemented (Yu *et al*., 2017). Here, we report the occurrence of a natural outbreak of ORFV infection in a goat farm in Anhui Province. The diseased goats showed severe scab symptoms, which is highly consistent with the clinical symptoms of common ORFV infection described previously.

At present, the detection of ORFV includes PCR, electron microscopic observation and cell culture, which are highly reliable (Kumar *et al*., 2014; Yu *et al*., 2017). According to published studies, the ORFV011 (B2L) and ORFV059 (F1L) genes, located in the highly conserved region of the genome, commonly serve as a marker to detect and systematically analyze the genetic relatedness of ORFV (Hosamani *et al*., 2006; Oem *et al*., 2013; Zhang *et al*., 2016). We designed specific primers for ORFV011 (B2L) and ORFV059 (F1L) genes and PCR results were positive. In addition, OFTu cells are often used for the isolation of ORFV (Kumar *et al*., 2014). Therefore, we prepared primary ovine fetal turbinate cells and infection with ORFV, resulted in a typical CPE phenomenon. At the same time, electron microscopy observation showed that virions like wool clusters were present. The result confirms that the outbreak in Anhui Province in 2018 was caused by ORFV.

To understand the source and transmission pathway of the virus strain to prevent and control the disease, we proceeded with phylogenetic analysis. The nt and aa sequence identities and phylogenetic tree showed that the isolates from Anhui Province were close to the ORFV reference strains, especially to the isolates from China, implying that the regional ORFV strains are prevalent in China. The same phenomenon was observed by Zhang *et al.* (2014) in a previous study. The phylogenetic analysis of ORFV011 (B2L) gene indicates that the AH/LA/18 strain has a common ancestor with Nantou strain, which was isolated from Taiwan. The ORFV059 (F1L) phylogenetic analysis tree showed that the AH/LA/18 strain is close to SDLC and SDTA, and found that the F1L amino acid sequence of Anhui isolates is 100% consistent with SDLC, which were isolated from East China.

Interestingly, this result is inconsistent. This could partly be attributed to the lack of ORFV (F1L) gene sequences from Nantou. Shandong flocks are adjacent to those in Anhui Province, a locality famous for sheep and goat breeding, and export of several mutton products (Zhang *et al.*, 2016). Nantou strain was isolated in 2007 from Nantou County, located in the center of Taiwan, which is separated by a strait from mainland China (Chan *et al.*, 2007). Therefore, owing to the geographical proximity of and commercial activities between the two provinces, the AH/LA/18 strain may be potentially transmitted from Shandong Province to Anhui, but this is yet to be confirmed. Moreover, in the past eleven years, it is also possible that the Nantou strain was introduced into mainland China, directly or indirectly, and then to Anhui Province. Of course, it is also necessary to carry out a detailed investigation on future trade in goat. In the end, we hypothesize that the AH/LA/18 strain may have originated from Nantou, or that it is a novel strain arising from genetic rearrangement of the Nantou and SDLC strains.

In summary, the determination of the precise method of transmission of the ORFV strain isolated in Anhui Province is challenging, because the introduction of ORFV may have been caused by the import of animals, sale of animal products, and other reasons. However, these data suggest that regional ORFV strains circulate in China. Therefore, the products of animal origin imported from affected areas should be carefully examined to prevent the spread of disease.

The results of the present study provide information on ORFV molecular epidemiology. To the best of our knowledge, our study is the first to systematically analyze the molecular characteristics of an orf strain isolated from goat farms in Anhui Province.

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References

Abdullah AA, Ismail MF, Balakrishnan KN, Bala JA, Hani H, Abba Y, Awang Isa MK, Abdullah FF, Arshad SS, Nazariah ZA, Abdullah R, Mustapha NM, Mohd-Lila MA (2015): Isolation and phylogenetic analysis of caprine Orf virus in Malaysia. Virus Disease 26, 255–259. https://doi.org/10.1007/s13337-015-0278-4

Al-Qattan MM (2011): Orf infection of the hand. J. Hand. Surg. Am. 36, 1855–1858. https://doi.org/10.1016/j.jhsa.2011.08.019

Bora DP, Barman NN, Das SK, Bhanuprakash V, Yogisharadhya R, Venkatesan G, Kumar A, Rajbongshi G, Khatoon E, Chakraborty A, Bujarbaruah KM (2012): Identification and phylogenetic analysis of orf viruses isolated from outbreaks in goats of Assam, a northeastern state of India. Virus Genes 45, 98–104. https://doi.org/10.1007/s11262-012-0740-y

Chan KW, Lin JW, Lee SH, Liao CJ, Tsai MC, Hsu WL, Wong ML, Shih HC (2007): Identification and phylogenetic analysis of orf virus from goats in Taiwan. Virus Genes 35, 705–712. https://doi.org/10.1007/s11262-007-0144-6

Chen H, Li W, Kuang Z, Chen D, Liao X, Li M, Luo S, Hao W (2017): The whole genomic analysis of orf virus strain HN3/12 isolated from Henan province, central China. BMC Vet. Res. 13, 260. https://doi.org/10.1186/s12917-017-1178-1

Duan C, Liao M, Wang H, Luo X, Shao J, Xu Y, Li W, Hao W, Luo S (2015): Identification, phylogenetic evolutionary analysis of GDQY orf virus isolated from Qingyuan City, Guangdong Province, southern China. Gene 555, 260–268. https://doi.org/10.1016/j.gene.2014.11.016

Fleming SB, Wise LM, Mercer AA (2015): Molecular genetic analysis of orf virus: a poxvirus that has adapted to skin. Viruses 7, 1505–1539. https://doi.org/10.3390/v7031505

Friebe A, Friederichs S, Scholz K, Janssen U, Scholz C, Schlapp T, Mercer A, Siegling A, Volk HD, Weber O (2011): Characterization of immunostimulatory components of orf virus (parapoxvirus ovis). J. Gen. Virol. 92, 1571–1584. https://doi.org/10.1099/vir.0.028894-0

Gelaye E, Achenbach JE, Jenberie S, Ayelet G, Belay A, Yami M, Loitsch A, Grabherr R, Diallo A, Lamien CE (2016): Molecular characterization of orf virus from sheep and goats in Ethiopia, 2008-2013. Virol. J. 13, 34. https://doi.org/10.1186/s12985-016-0489-3

Georgiades G, Katsarou A, Dimitroglou K (2005): Human ORF (Ecthyma contagiosum). J. Hand. Surg. Br. 30B, 409–411. https://doi.org/10.1016/J.JHSB.2005.03.010

Hosamani M, Bhanuprakash V, Scagliarini A, Singh RK (2006): Comparative sequence analysis of major envelope protein gene (B2L) of Indian orf viruses isolated from sheep and goats. Vet. Microbiol. 116, 317–324. https://doi.org/10.1016/j.vetmic.2006.04.028

Khalafalla AI, El-Sabagh IM, Al-Busada KA, Al-Mubarak AI, Ali YH (2015): Phylogenetic analysis of eight sudanese camel contagious ecthyma viruses based on B2L gene sequence. Virol. J. 12, 124. https://doi.org/10.1186/s12985-015-0348-7

Kumar N, Wadhwa A, Chaubey KK, Singh SV, Gupta S, Sharma S, Sharma DK, Singh MK, Mishra AK (2014): Isolation and phylogenetic analysis of an orf virus from sheep in Makhdoom, India. Virus Genes 48, 312–319. https://doi.org/10.1007/s11262-013-1025-9

Kummeneje K, Krogsrud J (1979): Contagious ecthyma (orf) in reindeer (Rangifer t tarandus). Vet. Rec. 105, 60–61. https://doi.org/10.1136/vr.105.3.60

Li H, Zhu X, Zheng Y, Wang S, Liu Z, Dou Y, Li H, Cai X, Luo X (2013): Phylogenetic analysis of two Chinese orf virus isolates based on sequences of B2L and VIR genes. Arch. Virol. 158, 1477–1485. https://doi.org/10.1007/s00705-013-1641-7

Li W, Ning Z, Hao W, Song D, Gao F, Zhao K, Liao X, Li M, Rock DL, Luo S (2012): Isolation and phylogenetic analysis of orf virus from the sheep herd outbreak in northeast China. BMC Vet. Res. 8, 229. https://doi.org/10.1186/1746-6148-8-229

Oem JK, Chung JY, Kim YJ, Lee KK, Kim SH, Jung BY, Hyun BH (2013): Isolation and characterization of orf viruses from Korean black goats. J. Vet. Sci. 14, 227–230. https://doi.org/10.4142/jvs.2013.14.2.227

Peralta A, Robles C, Martinez A, Alvarez L, Valera A, Calamante G, Konig GA (2015): Identification and molecular characterization of Orf virus in Argentina. Virus Genes 50, 381–388. https://doi.org/10.1007/s11262-015-1189-6

Schmidt C, Cargnelutti JF, Brum MC, Traesel CK, Weiblen R, Flores EF (2013): Partial sequence analysis of B2L gene of Brazilian orf viruses from sheep and goats. Vet. Microbiol. 162, 245–253. https://doi.org/10.1016/j.vetmic.2012.10.031

Spyrou V, Valiakos G (2015): Orf virus infection in sheep or goats. Vet. Microbiol. 181, 178–182. https://doi.org/10.1016/j.vetmic.2015.08.010

Sullivan JT, Mercer AA, Fleming SB, Robinson AJ (1994): Identification and characterization of an orf virus homologue of the vaccinia virus gene encoding the major envelope antigen p37K. Virology 202, 968–973. https://doi.org/10.1006/viro.1994.1420

Vikoren T, Lillehaug A, Akerstedt J, Bretten T, Haugum M, Tryland M (2008): A severe outbreak of contagious ecthyma (orf) in a free-ranging musk ox (Ovibos moschatus) population in Norway. Vet. Microbiol. 127, 10–20. https://doi.org/10.1016/j.vetmic.2007.07.029

Yu Y, Tan Q, Zhao W, Zhang X, Ma J, Wu Z, Zhu Z, Cui Y (2017): Characterization of an orf virus isolate from an outbreak in Heilongjiang province, China. Arch. Virol. 162, 3143–3149. https://doi.org/10.1007/s00705-017-3426-x

Zhang K, Liu Y, Kong H, Shang Y, Liu X (2014): Comparison and phylogenetic analysis based on the B2L gene of orf virus from goats and sheep in China during 2009-2011. Arch. Virol. 159, 1475–1479. https://doi.org/10.1007/s00705-013-1946-6

Zhang K, Xiao Y, Yu M, Liu J, Wang Q, Tao P, Liu S, Ning Z (2015): Phylogenetic analysis of three orf virus strains isolated from different districts in Shandong Province, East China. J. Vet. Med. Sci. 77, 1639–1645. https://doi.org/10.1292/jvms.15-0368