Molecular Epidemiological Analysis of Orf Viruses in Japanese Serows (*Capricornis crispus*)

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ABSTRACT

The orf virus infects sheep, goats, and Japanese serows (*Capricornis crispus*). It was first reported in Japanese serows in Iwate Prefecture in 1973, and has since spread among them throughout Japan. Because orf virus-susceptible livestock such as sheep and goats inhabits in Japan, there are the risk of transmission of Japanese serow-derived orf viruses to livestock. In this study, we determined nucleotide sequences (1,137 bp) of open reading frame (ORF) 11 of six strains isolated from Japanese serows and the Iwate strain isolated from sheep. Deduced amino acid sequences (378 aa) among them were 99–100% identical, indicating that ORF11 is highly conserved. Analysis of other regions of these viruses is needed to clarify the molecular epidemiological correlation among the Japanese strains.

Key words: Japanese serow, molecular epidemiological study, orf virus

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The orf virus belongs to the genus *Parapoxvirus*, subfamily *Chordopoxvirinae*, family *Poxviridae*, and mainly infects small ruminants such as sheep and goats, and can be transmitted to humans [1]. The mode of transmission is through contact infection such as infection from a wound [2, 3], and oral infection via virus-contaminated feed. The disease caused by the orf virus is known as contagious ecthyma or contagious pustular dermatitis. Outbreaks of contagious pustular dermatitis have been observed all over the world [4-8], and occur sporadically in Japan [9, 10]. The infection has also been confirmed in wild Japanese serows (*Capricornis crispus*), which are designated as a special natural monument of Japan [11]. Clinical signs tend to be more severe in Japanese serows than in sheep and goats [12]. Contagious pustular dermatitis of Japanese serows was first reported in Iwate Prefecture in 1973 [13]; subsequently, the disease has been spreading from Tohoku region to Kansai region [14]. A previous study reported

that nucleotide sequence identity in three open reading frames (ORFs), partial ORF11, complete ORF20, and complete ORF132 was highly conserved among 11 orf viruses isolated from sheep and Japanese serows [15]. These ORFs encode viral envelope, virus interferon resistance, and viral vascular endothelial growth factor, respectively. Besides, successful experiments on sheep infection using dermal lesions of a Japanese serow confirmed that contagious pustular dermatitis of wild Japanese serow was transmissible to sheep, indicating the risk of orf virus transmission among different wild and livestock animals [16]. In Japan, orf virus-susceptible livestock such as sheep and goats inhabits the whole country, there are the risk of transmission of Japanese serow-derived orf viruses to livestock. In previous reports, only a portion of ORF11 (554 bp) was compared [15, 17]; thus, we compared the full length of the sequence of ORF11 (1,137 bp) to obtain further genetic information about the strains of the orf virus isolated from sheep and Japanese serows. Further, phylogenetic analysis was also carried out.

Seven Japanese strains of the orf virus, Iwate [18] isolated

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Table 1 Japanese and reference strains of orf virus used in this study

Strain	Host	Year of isolation	Country	Prefecture
Iwate	Sheep	1970	Japan	Iwate
S-1	Japanese serow	1985	Japan	Gifu
GHF	Japanese serow	1999	Japan	Gifu
R-1	Japanese serow	1999	Japan	Toyama
Aichi	Japanese serow	2000	Japan	Aichi
GE	Japanese serow	2007	Japan	Gifu
IJS081	Japanese serow	2008	Japan	Ishikawa
NZ2	Sheep	1982	New Zealand	
IA82	Sheep	1982	USA	
GO	Goat	2012	China	
YX	Goat	2012	China	

Iwate	1	MWPFSSIPGLGDCRVVETLPAEVLASLAQGNMSTLDCFTAIAESAKKFLYICSFCCNLSSSTKEGVDVKDKLCTLAKEGVNVTLLVDVQSKDKDADELREAGVNYKVKVSTKEGVGNLLGSFWLSDAGHWY	130
GE	1		130
S-1	1		130
R-1	1		130
GHF	1	T.....	130
IJS081	1		130
Aichi	1		130
NZ2	1	D.....	130
IA82	1	V.....D.....A.....T.N.....	130
YX	1	V.....D.....A.....R.....I.T.....	130
GO	1	V.....D.....A.....R.....	130
Iwate	131	VGSASLTGGSVSTIKNLGLYSTNKHAWDLNRYNTFYSMIVEPKVPFTRLCCAVVTPPTATNFHLDHSGGGVFFSDSPERFLGFYRTLDEDLVLHRIENAKNSIDLSSLMSVPVIKHASAVEYWPRIIDA	260
GE	131		260
S-1	131		260
R-1	131		260
GHF	131		260
IJS081	131		260
Aichi	131		260
NZ2	131	I.....	260
IA82	131	N.....G.....	260
YX	131	N.....G.....	260
GO	131	N.....G.....	260
Iwate	261	LLRAAIDRGVVRVVIITEWKNDPLSVSAARSLDDFGVGSVDMSVRKFVVPGRDDAANNTKLLIVDDTFAHLTVANLDGTHYRYHAFVSVNAEKGDIVKDL SAVFERDWRSEFCCKPIN*	379
GE	261		379
S-1	261		379
R-1	261		379
GHF	261		379
IJS081	261		379
Aichi	261	R.....	379
NZ2	261	N.....	379
IA82	261	N.....	379
YX	261		379
GO	261		379

Fig. 1 Alignment of deduced amino acid sequences derived from ORF11. Amino acid identical to the Iwate strain at the given positions are represented by dots.

from sheep and Aichi [19], GE [15], GHF [19], IJS081 [20], S-1 [10], and R-1 [19] isolated from wild Japanese serows, were used in this study (Table 1). Viruses were propagated in fetal lamb lung cells (kindly provided by Dr. H. Sentsui, Nihon University, Japan) and maintained in Eagle's minimum essential medium (Wako, Osaka, Japan) supplemented with 10 % fetal bovine serum (PAA Laboratories, Pasching, Austria).

DNA was extracted from virus-infected cells using a DNeasy

Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Polymerase chain reactions (PCRs) were carried out using the Veriti thermal cycler (Applied Biosystems, Foster City, CA, U.S.A.). PCR for ORF11 encoding viral envelope was conducted as follows. PCR reaction mixture was prepared to a final volume of 50 µl containing 0.4 µM each of primers, ORF11F (5'- CAA ATC AAG TCC GAG CTC CAG -3'), and ORF11R (5'- GAC GGC CAA TAG CGG GGC TT

Table 2 Deduced amino acid sequence identities ORF11 (%)

Strain	Iwate	S-1	R-1	GHF	Aichi	GE	IJS081	NZ2	IA82	GO	YX
Iwate											
S-1	100										
R-1	100	100									
GHF	99	99	99								
Aichi	99	99	99	99							
GE	100	100	100	99	99						
IJS081	100	100	100	99	99	100					
NZ2	98	98	98	98	98	98	98				
IA82	97	97	97	97	97	97	97	97			
GO	98	98	98	98	98	98	98	97	98		
YX	97	97	97	97	97	97	97	97	98	99	

-3'), 800 μ M dNTP mix, 10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 1U AmpliTaq Gold DNA polymerase (Applied Biosystems), and 5–100 ng of extracted DNA from virus-infected cells. Thermal cycling was performed for 9 min at 95°C, followed by 40 cycles at 94°C for 1 min, at 55°C for 1 min, and at 72°C for 1.5 min, with a final extension at 72°C for 7 min.

For sequencing, PCR products were purified using a QIAquick PCR Purification Kit (Qiagen), and nucleotide sequences were determined by direct sequencing using a BigDye Terminator Cycle Sequencing Kit Ver. 3.1 (Applied Biosystems). Phylogenetic analysis was performed using MEGA X Version 10.0.5 program [21]. A phylogenetic tree was constructed using the method of maximum likelihood with 1,000 bootstrap replicates. Sequences and phylogenetic analysis were compared with the reference strains of orf viruses (Table 1).

Previous studies analyzed only a portion of ORF11 (554 bp) [15, 17]. To further investigate the molecular epidemiological correlation between the orf viruses of sheep and Japanese serows, we determined nucleotide sequences (1,137 bp) of full length of ORF11 from all of the seven Japanese strains of orf viruses (Fig. 1). Deduced amino acid identity between the Iwate strain and the Japanese serow strains was found to be 99–100 %, whereas the amino acid identity between the Iwate strain and foreign strains (NZ2, IA82, GO, and YX) was 97–98 % (Table 2). Furthermore, the phylogenetic tree of ORF11 indicated that the Iwate strain belonged to the same cluster of Japanese serow strains (Fig. 2), but belonged to another cluster of foreign strains. Our data indicated that full length

Fig. 2 Phylogenetic tree based on deduced amino acid sequences derived from ORF11. The percentage bootstrap values of more than 80% (1,000 replications) are indicated above the internal nodes. The scale bar beneath the tree stands for amino acid substitution per site.

of ORF11 is highly conserved among Japanese orf viruses. In addition, ORF11 is located in the central region of the orf virus, which has been reported as a relatively conserved region in parapoxviruses, such as bovine papular stomatitis virus and orf virus [22]. Although ORF11 would be suitable for species-distinguishment in parapoxviruses, it would not be suitable for phylogenetic analysis of orf viruses. Hence, to prove this hypothesis, an analysis of the variable regions, which might locate in other than the central region of the genomes of these viruses will be needed.

To conclude, the results of this study reveal that the full length of ORF11 is highly conserved among Japanese orf viruses. Further studies will be needed to clarify genetic correlation between orf viruses from sheep and Japanese serows.

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REFERENCES

1. Buller RM, Arif BM, Black DN, Dumbell KR, Esposito JJ, Lefkowitz EJ, Mcfadden G, Moss B, Mercer AA, Moyer RW, Skinner MA, Trioathy DN. 2005. Family *Poxviridae*. In *Virus Taxonomy Eighth Report of the International Committee on Taxonomy of Viruses* (Fauquet CM, Mayo MA, Manilof J, Dessselberger U, Ball LA ed.), pp.117-133. Elsevier Academic Press. London.
2. Diven DG. 2001. An overview of poxviruses. *J Am Acad Dermatol* 44: 1-16.
3. Fleming SB, Wise LM, Mercer AA. 2015. Molecular genetic analysis of orf virus: a poxvirus that has adapted to skin. *Viruses* 7: 1505-1539.
4. 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.
5. Chi X, Zeng X, Hao W, Li M, Li W, Huang X, Wang S, Luo S. 2013. Heterogeneity among orf virus isolates from goats in Fujian Province, Southern China. *PLoS One* 8: e66958.
6. 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.
7. 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.
8. 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.
9. Suzuki Y, Sugimura M, Atoji Y, Minamoto N, Kinjo T. 1986. Widespread of parapox infection in wild Japanese serows, *Capricornis crispus*. *Jpn J Vet Sci* 48: 1279-1282.
10. Suzuki T, Minamoto N, Sugiyama M, Kinjo T, Suzuki Y, Sugimura M, Atoji Y. 1993. Isolation and antibody prevalence of a parapoxvirus in wild Japanese serows (*Capricornis crispus*). *J Wildl Dis* 29: 384-389.
11. Inoshima Y, Yamamoto Y, Takahashi T, Shino M, Katsumi A, Shimizu S, Sentsui H. 2001. Serological survey of parapoxvirus infection in wild ruminants in Japan in 1996-9. *Epidemiol Infect* 126: 153-156.
12. Inoshima Y, Ishiguro N. 2010. Molecular and biological characterization of vascular endothelial growth factor of parapoxviruses isolated from wild Japanese serows (*Capricornis crispus*). *Vet Microbiol* 140: 63-71.
13. Masui M. 1977. *Anima* 50: 88.
14. Inoshima Y. 2013. Parapoxvirus infection in wild Japanese serows (*Capricornis crispus*). *J Jpn Vet Med Assoc* 66: 557-563.
15. Inoshima Y, Ito M, Ishiguro N. 2010. Spatial and temporal genetic homogeneity of orf viruses infecting Japanese serows (*Capricornis crispus*). *J Vet Med Sci* 72: 701-707.
16. Masegi T, Hori M, Sakai H, Inoshima Y, Yanai T. 2002. Parapoxvirus infection in wild Japanese serow -A possibility of infection from wild serow to sheep-. *J Zoo Wildl Med* 7: 39-43.
17. Inoshima Y, Murakami K, Yokoyama T, Sentsui H. 2001. Genetic heterogeneity among parapoxviruses isolated from sheep, cattle and Japanese serows (*Capricornis crispus*). *J Gen Virol* 82: 1215-1220.
18. Kumagai T, Shimizu M, Ito, Y, Konno S, Nakagawa N, Sato K, Mukainakano K, Ohta H. 1971. Contagious pustular dermatitis of sheep. *71st Meeting of the Japanese Society of Veterinary Science*. Tokyo.
19. Inoshima Y, Murakami K, Wu D, Sentsui H. 2002. Characterization of parapoxviruses circulating among wild Japanese serows (*Capricornis crispus*). *Microbiol Immunol* 46: 583-587.
20. Ito M, Hayakawa Y, Shintani E, Inoshima Y. 2009. Molecular characterization of parapoxviruses from infected wild Japanese serows. *J Zoo Wildl Med* 14: 103-106.
21. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular evolutionary genetics analysis across computing

platforms. *Mol Biol Evol* 35: 1547-1549.

22. Delhon G, Tulman ER, Afonso CL, Lu Z, de la Concha-Bermejillo A, Lehmkuhl HD, Piccone ME, Kutish GF, Rock DL.

2004. Genomes of the parapoxviruses ORF virus and bovine papular stomatitis virus. *J Virol* 78: 168-177.

研究短報 ウイルス学

ニホンカモシカに感染しているオーフウイルスの分子疫学的解析

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要 約

オーフウイルスは、羊や山羊に感染し、日本ではニホンカモシカへの感染も報告されている。全国のニホンカモシカ由来オーフウイルス6株の分子疫学的解析を行った。オープンリーディングフレーム11 (ORF11) 全長の推定アミノ酸配列は99-100%同一であり、高度に保存されていることが示された。ORF11はパラポックスウイルスの種の同定には適しているが、オーフウイルス間の分子疫学的解析のためには他領域の解析が必要と考えられた。

キーワード：オーフウイルス, ニホンカモシカ, 分子疫学解析

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