Molecular Characterization of Parapoxviruses from Infected Wild Japanese Serows (*Capricornis crispus*) in Ishikawa Prefecture

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石川県のニホンカモシカ (*Capricornis crispus*) に感染している パラポックスウイルスの遺伝学的解析

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ABSTRACT. Cases of suspected parapoxvirus infection were observed in wild Japanese serows (*Capricornis crispus*) in different areas of Hakusan in Ishikawa Prefecture in 2008. Polymerase chain reaction (PCR) and sequence analyses were conducted, and a specific 594-bp fragment was amplified by parapoxvirus-specific PCR from the lesions of two serows from distinct areas within the Prefecture. Parapoxviruses were isolated from the lesions of two serows and were identified as orf virus, designated IJS081 and IJS087. Partial sequences of the viral envelope gene were determined from the isolated IJS081 and IJS087. The sequences were identical to each other and to the orf virus S-1 strain that was isolated from a Japanese serow in the neighboring Gifu Prefecture in 1985. These results suggest that the infections in Japanese serows in different areas of Hakusan were caused by the same or genetically similar orf virus. Moreover, the sequence of the viral envelope gene in orf viruses in Japanese serows in this area seems to have been stable for more than 20 years.

Keywords : Hakusan, Japanese serow, orf virus, parapoxvirus, sequence

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INTRODUCTION

Parapoxviruses cause papules, nodules, and scabs in the skin around the mouth and teats of affected ruminants, cattle, sheep, and goats [1]. Parapoxvirus infection has a low fatality rate in animals, but the lesions around the mouth or on teats can progress to ulcers in some severe cases. The genus *Parapoxvirus* in the family *Poxviridae* has four members, orf virus (ORFV), bovine papular stomatitis virus, pseudocowpox virus, and parapoxvirus of red deer in New Zealand. Although all four members are serologically cross-reactive and cause similar histopathological lesions in the skin, they can be distinguished by polymerase chain reaction with restriction fragment length polymorphism (PCR-RFLP) [2, 3].

Parapoxvirus infection is also observed in wild Japanese serows (*Capricornis crispus*) in Japan. The disease is characterized by papules, pustules, and scabs around the lips, nose, and eyes. In 2008, cases of suspected parapoxvirus infection were observed in wild Japanese serows in two

different areas of Hakusan in Ishikawa Prefecture (Fig. 1). As a result of the widely spread habitat and increasing population of wild Japanese serows in Ishikawa Prefecture [4], the likelihood of finding diseased serows is great. However, the relationship between the infections in different areas as well as other prefectures remains unclear.

In this study, using PCR, we diagnosed two cases of infection in Japanese serows as parapoxvirus infection, and the viruses were classified as ORFV. Moreover, we conducted sequence analysis of the viral envelope gene of the viruses from both infections, and compared this sequence to that of an ORFV isolated in 1985 from a serow in a neighboring prefecture.

MATERIALS AND METHODS

Animals

Two dead Japanese serows with purulent scabs on the lips, nose, and eyes (Fig. 2) were brought to our facility on January 30, 2008 (serow No. 1) and July 15, 2008 (serow No. 2). Serow No. 1 was found in a weakened condition with

Fig. 1 A map showing the Hakusan area and the locations where Japanese serows No. 1 and No. 2 were found. (A) The discovery locations are indicated by dots. (B) Enlargement of discovery locations. Serows No. 1 and No. 2 were found in Hakusan-shi and Nomi-shi, respectively, approximately 11 km apart.

inability to eat due to the severe ulcers around the mouth in Hakusan-shi (Fig. 1) on January 25, 2008. It was given medical treatment by a veterinarian, but died after four days. Serow No. 2 was severely injured after an automobile accident in Nomi-shi (Fig. 1) on July 14, 2008, and was subsequently euthanized.

Fig. 2 Parapoxvirus infection of a Japanese serow (No. 2).

Virus isolation

Primary fetal lamb lung (FLL) cells and bovine testis (BT) cells were cultured in Eagle's minimum essential medium (Nissui, Tokyo, Japan) supplemented with 0.295% tryptose phosphate broth (Difco, MI, USA), 60 mg/l of kanamycin and 5% fetal bovine serum at 37 °C in a humidified atmosphere of 5% CO₂ in air. Suspensions containing 10% samples of lesions of the lips in medium were inoculated into FLL or BT cells. The cells were then passaged until cytopathic effects (CPE) were observed.

PCR

DNA was extracted from suspensions containing 10% samples of the lesions or cells showing CPE using a QIAamp DNA mini kit (Qiagen, Hilden, Germany). PCR for parapoxvirus was carried out using a parapoxvirus-specific primer pair, PPP-1 and PPP-4, as described previously [2]. These primers detect viral envelope gene of all members of the genus *Parapoxvirus* and the expected size of amplified product is 594 bp.

RFLP

Amplified PCR product was digested with restriction endonucleases *Drd* I, *Xmn* I, *Pfl* MI, and *Hinc* II for the identification of the parapoxvirus species [3].

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Fig. 3 PCR detection of parapoxvirus and PCR-RFLP identification of parapoxvirus species. (A) PCR. A specific 594-bp DNA sequence was amplified from the skin lesions of a Japanese serow (No. 1). (B) PCR-RFLP. PCR product was digested with restriction enzymes, *Drd* I, *Xmn* I, *Pfl* MI, and *Hinc* II, for identification of parapoxvirus species. As a control, PCR product was treated without restriction enzyme in reaction buffer. Digestion was observed only with *Drd* I, indicating that the infecting parapoxvirus was ORFV.

Sequencing

Partial nucleotide sequences (554 bp) of the viral envelope gene were determined by direct sequencing using PCR products. Sequences were obtained from both strands for verification and compared to homologous sequence from other parapoxvirus.

RESULTS

Virus isolation

The FLL cells inoculated with the sample from serow No. 1 and the BT cells inoculated with the sample from serow No. 2 displayed CPE after two passages and the isolated virus was named IJS081 and IJS087 (Ishikawa Japanese serow 2008 January and July), respectively.

PCR and PCR-RFLP

From the lesion emulsions of serows No. 1 and No. 2, and from FLL and BT cells showing CPE, a specific 594-bp fragment of the envelope gene was amplified by PCR (Fig. 3A). The amplified PCR product was digested by *Drd* I, but not by *Xmn* I, *Pfl* MI, and *Hinc* II (Fig. 3B). This RFLP pattern is indicative

of ORFV, and the isolated IJS081 and IJS087 were classified as ORFV with the results of following sequence analysis.

Sequencing

A partial nucleotide sequence of the viral envelope gene was determined from the PCR product from both serows No. 1 and No. 2. The sequences were identical to each other (data not shown) and also identical to the S-1 strain of ORFV (Accession No. AB044796) [3], which was isolated in 1985 from a Japanese serow in the neighboring Gifu Prefecture [5].

DISCUSSION

In January and July of 2008, two Japanese serows showing purulent scabs on the lips, nose, and eyes were preserved and analyzed for pathogens. We diagnosed the disease as contagious pustular dermatitis by parapoxvirus infection and the causative agent was identified as ORFV. Sequence analysis revealed that partial nucleotide sequences of the viral envelope gene from the two Japanese serows were identical to each other and to the ORFV S-1 strain. These results indicate that genetically identical or closely related ORFV infected the two

serows separately, and that the virus is likely to be genetically similar or identical to the ORFV S-1 strain isolated in 1985 from a Japanese serow [5]. The mutation frequency of the genome of DNA viruses, including ORFV, is generally less than that of RNA virus genomes, which is mainly due to the fidelity of the DNA polymerase. It is estimated that the mutation frequency of RNA viruses is $0.2-25 \times 10^{-5}$ mutations/base/replication cycle, which is 10^3-10^5 times more than that of DNA-based haploid microbes [6, 7]. The complete sequence of the viral envelope gene of Indian ORFVs from sheep and goats shows 97–98 % sequence identity with that of other ORFVs from around the world [8]. We previously investigated the sequence of the envelope gene of ORFVs from Japanese serows and revealed that the sequence, from the same region in the envelope gene as in this study, is identical among 10 ORFVs isolated from 1999 to 2001 from Gifu, Toyama, Aichi, Nagano, Fukushima, and Tochigi Prefectures [9]. These results suggest that the viral envelope sequence is highly conserved in ORFVs, especially in Japanese ORFVs, and that it has been stable for more than 20 years among ORFVs in Japanese serows.

Moreover, the envelope sequence is identical to the ORFV Iwate strain isolated from sheep [9] and ORFV isolated from Japanese serows has been shown, experimentally, to infect sheep [10, 11], suggesting that the spread of parapoxvirus infection from Japanese serows to sheep is possible. The increasing serow populations and the continuing expansion of their habitat are likely to increase the risk of transmission of viruses from diseased Japanese serows to domestic animals.

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要 約

2008年石川県白山地域の異なる2地区で保護され、パラポックスウイルス感染症の疑いで病性鑑定を実施したニホンカモシカ2頭についてウイルス分離、およびウイルスエンベロープ領域の塩基配列解析を実施した。PCRでパラポックスウイルス特異的遺伝子が検出され、遺伝子解析によりオーフウイルスと同定した。2頭それぞれの病変部からウイルスが分離され、IJS081株とIJS087株と命名した。分離した2株のPCR産物から塩基配列を決定し比較したところ、別時期、かつ異なる地域で発症した2頭から分離したウイルスでありながら同じ配列を有していた。さらに、1985年に隣の岐阜県のニホンカモシ

カから分離したS-1株とも完全に一致していた。以上より、白山地域一帯のニホンカモシカに感染しているパラポックスウイルスは、遺伝的に同一あるいは極めて近いウイルスであり、さらにエンベロープ領域は20年以上にわたり安定的に維持されていることが示唆された。

キーワード：白山、ニホンカモシカ、オーフウイルス、パラポックスウイルス、シークエンス

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