

Genetic heterogeneity among parapoxviruses isolated from sheep, cattle and Japanese serows (*Capricornis crispus*)

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Standard strains of four parapoxviruses and seven unclassified Japanese strains isolated from sheep, cattle and wild Japanese serows (*Capricornis crispus*) were compared molecularly. Restriction fragment length polymorphism (RFLP) analysis of viral DNA, indirect immunofluorescence assays using monoclonal antibodies, partial nucleotide sequencing of the envelope gene, phylogenetic analysis and PCR-RFLP were carried out. These analyses revealed that the parapoxviruses were divided into four groups and the region sequenced in this study was highly conserved within each group. Each of the Japanese isolates was classified into one of these groups. These findings also indicated that parapoxvirus infections among wild Japanese serows seem to be caused by at least two different parapoxviruses, bovine papular stomatitis virus and orf virus. The methods presented here are useful for genetic characterization and classification of parapoxviruses.

The genus *Parapoxvirus* in the family *Poxviridae* has four members, orf virus (ORFV), bovine papular stomatitis virus (BPSV), pseudocowpox virus (PCPV) and parapoxvirus of red deer in New Zealand (PVNZ). Parapoxvirus infections are widespread in ruminants worldwide. Parapoxviruses generally induce mild papular dermatitis around the mouth, teats and skin of affected animals such as sheep, goats, cattle and some wild species (Esposito *et al.*, 1995; Fenner, 1996; Mayr & Büttner, 1990*a, b, c*; Mercer *et al.*, 1997; Moss, 1996; Robinson & Lyttle, 1992). The lesions progress from papule/vesicle to pustule and scab formation over a period of about 1 month (Okada *et al.*, 1986; Wheeler & Cawley, 1956). In some severe

cases of stomatitis or dermatitis on teats, the lesions interfere with feeding or milking and these animals become unproductive. The viruses occasionally infect humans after close contact with skin lesions of infected animals or handling virus-contaminated materials. Milker's nodule is known as one of the diseases in humans caused by parapoxvirus (Fenner, 1996; Mayr & Büttner, 1990*b*; Memar & Tying, 1995; Robinson & Lyttle, 1992), and the infections are therefore classed as zoonoses.

In Japan, serological surveys have revealed that the morbidity of parapoxvirus infection is very high in sheep and cattle (Kuroda *et al.*, 1999; Sentsui *et al.*, 2000). Although several parapoxviruses have been isolated from sheep and cattle with or without clinical symptoms, these isolates have not been classified biologically and molecularly. Moreover, parapoxvirus infections in wild Japanese serows (*Capricornis crispus*) have been prevalent continuously in various areas since the 1970s (Inoshima *et al.*, 1999, 2000*b*, 2001) and experimental transmission of parapoxvirus from the Japanese serow to cattle, sheep and goats has also succeeded (Ogino *et al.*, 1996; Okada *et al.*, 1986). The parapoxviruses circulating among Japanese serows have not yet been identified and the relationship between the virus in domestic animals and that in Japanese serows remains unclear. PVNZ was isolated from red deer in New Zealand and classified as a new parapoxvirus (Robinson & Mercer, 1995). Thus, it is possible that a new parapoxvirus may be distributed among wild Japanese serows.

The classification of parapoxviruses was formerly based on natural host range, clinical symptoms and serology (Robinson & Lyttle, 1992). However, some ORFVs were isolated from cattle and chamois rather than sheep (Robinson & Lyttle, 1992) and one strain was isolated from peripheral blood leukocytes of cattle rather than from teats or around the mouth (Sentsui *et al.*, 1999). Moreover, they cross-react antigenically with each other and there are so far no established serological guidelines for their classification (Lard *et al.*, 1991; Rosenbusch & Reed, 1983; Wittek *et al.*, 1980). More recently, classification has been attempted according to restriction endonuclease analysis and DNA-DNA hybridization of viral DNA (Esposito *et al.*, 1995; Fenner, 1996; Mercer *et al.*, 1997; Moss, 1996; Robinson & Lyttle, 1992). Virus isolation is required for such studies; however, virus isolation is time-consuming and laborious and

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The nucleotide sequence data reported in this study will appear in DDBJ/EMBL/GenBank under accession numbers AB044792–AB044801.

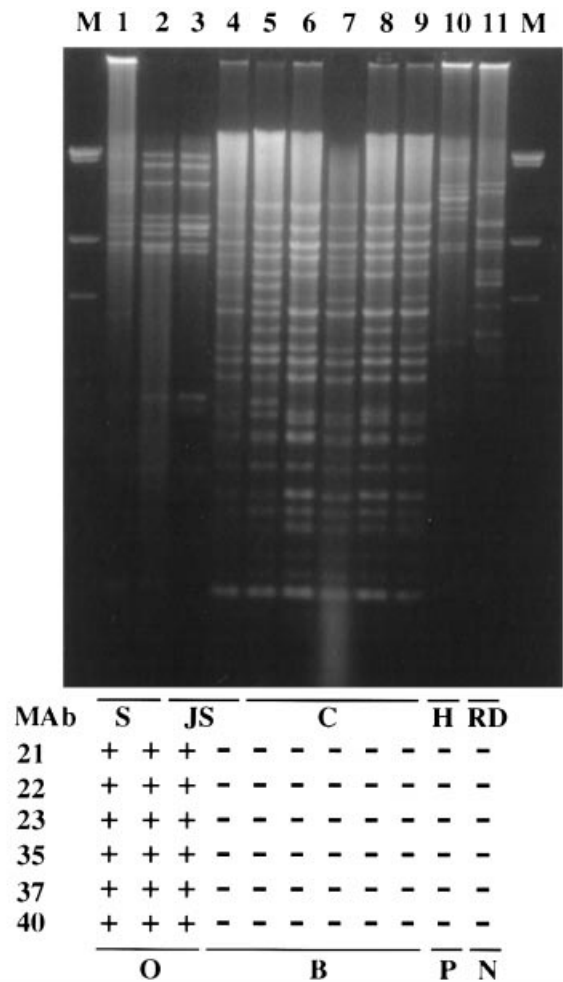


Fig. 1. Comparison of virus DNA digested with restriction endonuclease *KpnI* and reactivity to MAbs by immunofluorescence assay. Lanes: M, markers; 1, ORFV strain NZ2; 2, Iwate; 3, S-1; 4, Ishikawa-S; 5, BPSV strain V660; 6, Aomori; 7, Chiba; 8, V94; 9, Ishikawa-B; 10, PCPV strain VR634; 11, PVNZ strain DPV. The biological source of each strain is indicated as: S, sheep; JS, Japanese serows; C, cattle; H, humans; RD, red deer in New Zealand. Patterns corresponding to the four parapoxvirus species are indicated by: O, ORFV; B, BPSV; P, PCPV; N, PVNZ.

is sometimes unsuccessful. In many viruses, comparison of nucleotide and amino acid sequences of virus genomes and phylogenetic analyses have been used effectively for molecular characterization and classification. Since no sequencing or phylogenetic analyses have been carried out on parapoxviruses other than ORFV, the relationships between the four parapoxvirus groups remain unclear and the present criteria for classification into the four groups are still problematic.

In this study, we investigated genetic heterogeneity among unclassified Japanese strains of parapoxvirus isolated from sheep, cattle and Japanese serows and also four standard strains in the genus *Parapoxvirus*. Moreover, we attempted to develop a method for genetic classification of parapoxviruses.

Primary foetal bovine muscle (FBM) cells were used for virus propagation. Eleven strains of parapoxvirus were used in

this study. ORFV strain NZ2 (Robinson *et al.*, 1982), BPSV strain V660 (Menna *et al.*, 1979), PCPV strain VR634 (Friedman-Kien *et al.*, 1963) and PVNZ strain DPV (Horner *et al.*, 1987; Robinson & Mercer, 1995) were used as standard strains of the four members of the genus *Parapoxvirus*. The four strains were kindly provided by A. A. Mercer (University of Otago, Dunedin, New Zealand). The Iwate strain was isolated from a sheep (Kumagai *et al.*, 1971). Strains Aomori (Kumagai *et al.*, 1976), Chiba (Kuroda *et al.*, 1999), V94 (Sentsui *et al.*, 1999) and Ishikawa-B (Kuroda *et al.*, 1999) were isolated from cattle. Strains S-1 (Suzuki *et al.*, 1993) and Ishikawa-S (Yata *et al.*, 1996) were isolated from wild Japanese serows. Strains Ishikawa-B, Ishikawa-S and S-1 were kindly provided by T. Murakami (Nanbu Livestock Hygiene Service Station, Ishikawa, Japan) and N. Minamoto (Gifu University, Gifu, Japan).

Firstly, the strains were compared by restriction fragment length polymorphism (RFLP) analysis with the restriction endonuclease *KpnI* (Fig. 1), which is thought to be useful for molecular characterization of parapoxviruses (Mercer *et al.*, 1997; Robinson & Lyttle, 1992). Viral DNA was extracted from infected FBM cells by alkaline lysis followed by phenol extraction (Inoshima *et al.*, 2000a) or from the purified virus by sodium diatrizoate gradients (Inoshima *et al.*, 1999). The four standard strains showed different DNA patterns. The Iwate strain and ORFV strain NZ2 showed the same restriction profile. All four strains isolated from cattle (Aomori, Chiba, V94 and Ishikawa-B) showed similar patterns to each other and the patterns resembled that of BPSV V660, even though some of these strains were isolated from teats or leukocytes rather than from around the mouth. Interestingly, the patterns of the two strains from Japanese serows were significantly different from each other. S-1 was similar to ORFV whereas Ishikawa-S was BPSV. The possibility of virus contamination was ruled out by RFLP of viral DNA with *SalI*, whereby different DNA patterns were shown among Japanese strains (data not shown).

Next, all 11 strains were tested for reactivity to six monoclonal antibodies (MAbs 21, 22, 23, 35, 37 and 40) against ORFV (Ueda, 1996) by an indirect immunofluorescence assay. These MAbs were kindly provided by N. Ueda (University of Otago, Dunedin, New Zealand) and K. Miyamoto (Wakayama Medical College, Wakayama, Japan). Strains NZ2, Iwate and S-1 reacted with all six MAbs; none of the other strains, including Ishikawa-S, reacted with any of the MAbs (Fig. 1). Similar to the RFLP analysis using *KpnI*, the two strains from Japanese serow showed different characteristics.

Next, partial nucleotide sequences (554 bp) of the envelope gene were determined by PCR amplification (Inoshima *et al.*, 2000b) and direct sequencing. Sequences were obtained from both strands of each PCR product for verification. The sequences obtained in this study were submitted to DDBJ/EMBL/GenBank under accession numbers AB044792–AB044801. The sequence of ORFV strain NZ2 was obtained from accession number U06671. Neither deletions nor

Table 1. Percentage nucleotide and deduced amino acid identities between parapoxvirus envelope genes

Nucleotide sequence identities are presented above the diagonal and amino acid sequence identities below the diagonal.

Strain	ORFV			BPSV					PCPV	PVNZ	
	1	2	3	4	5	6	7	8	9	10	11
1. NZ2		99.1	99.1	83.8	83.6	83.8	83.8	83.8	83.8	95.3	82.3
2. Iwate	98.4		100	84.1	83.9	84.1	84.1	84.1	84.1	94.8	82.3
3. S-1	98.4	100		84.1	83.9	84.1	84.1	84.1	84.1	94.8	82.3
4. Ishikawa-S	83.2	84.2	84.2		98.9	100	100	100	100	85.0	86.3
5. V660	83.2	84.2	84.2	100		98.9	98.9	98.9	98.9	84.5	86.5
6. Aomori	83.2	84.2	84.2	100	100		100	100	100	85.0	86.3
7. Chiba	83.2	84.2	84.2	100	100	100		100	100	85.0	86.3
8. V94	83.2	84.2	84.2	100	100	100	100		100	85.0	86.3
9. Ishikawa-B	83.2	84.2	84.2	100	100	100	100	100		85.0	86.3
10. VR634	95.1	93.5	93.5	82.6	82.6	82.6	82.6	82.6	82.6		83.2
11. DPV	79.9	79.9	79.9	86.4	86.4	86.4	86.4	86.4	86.4	81.5	

insertions in the nucleotide sequences were found between the strains. Overall nucleotide identities ranged from 82.3 to 100% (Table 1). High levels of identity were observed among isolates belonging to the same member of the genus, regardless of their geographical origin, host or year of isolation. The four Japanese isolates from cattle were 100% identical.

The deduced amino acid sequences were aligned and compared (Fig. 2*a*; Table 1). Overall amino acid identity in the region ranged from 79.9 (between ORFVs and PVNZ) to 100% (ORFVs and BPSVs). Among the four standard strains, the identity ranged from 79.9 (between ORFV and PVNZ) to 95.1% (between ORFV and PCPV), indicating that there was a closer relationship in the region between ORFV and PCPV than among the other parapoxviruses. These results corresponded with previous results of Southern blot analysis (Inoshima *et al.*, 2000*b*). Surprisingly, two strains (Iwate and S-1) showed 100% amino acid identity and six strains (V660, Ishikawa-S, Aomori, Chiba, V94 and Ishikawa-B) were completely homologous with each other, even though they were isolated independently geographically, biologically and chronologically. This region is a homologue of the major envelope antigen of vaccinia virus and one of a limited number of ORFV proteins to which sheep mount a strong antibody response and which stimulate lymphocytes (Sullivan *et al.*, 1994). The region also encodes an envelope protein in other viruses belonging to the family *Poxviridae*, such as molluscum contagiosum virus (Blake *et al.*, 1991), fowlpox virus (Calvert *et al.*, 1992), swinepox virus (Bárcena *et al.*, 2000) and sheeppox virus (H. G. Heine, M. F. Rudd, A. J. Foord and D. B. Boyle, unpublished data). Generally, the virus envelope antigen is exposed to immunological pressure from the host and tends to be variable. Thus, our results suggest that this region is a highly conserved genetic marker of each parapoxvirus.

Phylogenetic analysis was performed by using the un-

weighted pair group method using arithmetic averages (UPGMA) and a tree was constructed with GENETYX-MAC version 9.0 (Software Development Co.). On the basis of the derived tree, the parapoxviruses were divided into four phylogenetic clusters (Fig. 2*b*). The cluster of ORFV included Iwate and S-1. The cluster of BPSV included Ishikawa-S and the four strains from cattle.

The nucleotide sequence data allowed us to search for restriction enzyme recognition sites specific to a particular parapoxvirus. *DrdI*, *XmnI*, *PfI*MI and *HincII* were each able to differentiate one parapoxvirus from the others (Fig. 2*c*). High conservation of the envelope gene in each cluster may make it possible to differentiate parapoxviruses by PCR-RFLP. Taken together, these results indicated that Iwate and S-1 belong to ORFV whereas the other strains (Ishikawa-S, Aomori, Chiba, V94 and Ishikawa-S) belong to BPSV.

Since the lesions of the diseases caused by each of the four parapoxviruses exhibit almost the same phenotype and they cross-react antigenically, classification of parapoxviruses by natural host range and clinical symptoms is not definite and serological tests are not effective. This study represents the first phylogenetic analysis of the genus *Parapoxvirus* and demonstrates genetic heterogeneity within the genus. According to the criteria of parapoxvirus classification on the basis of host and the location of the lesion, Chiba and Ishikawa-B, isolated from teats, would be classified into PCPV and V94, isolated from peripheral blood leukocytes, could not be classified. However, Chiba, Ishikawa-B and V94 were classified into BPSV. Ishikawa-S, which was isolated from the Japanese serow, was also classified into BPSV. Such contradictions were also observed with some isolates of ORFV isolated from cattle and chamois rather than sheep (Robinson & Lyttle, 1992). These findings suggest that classification of parapoxviruses by host and clinical symptoms does not always reflect the

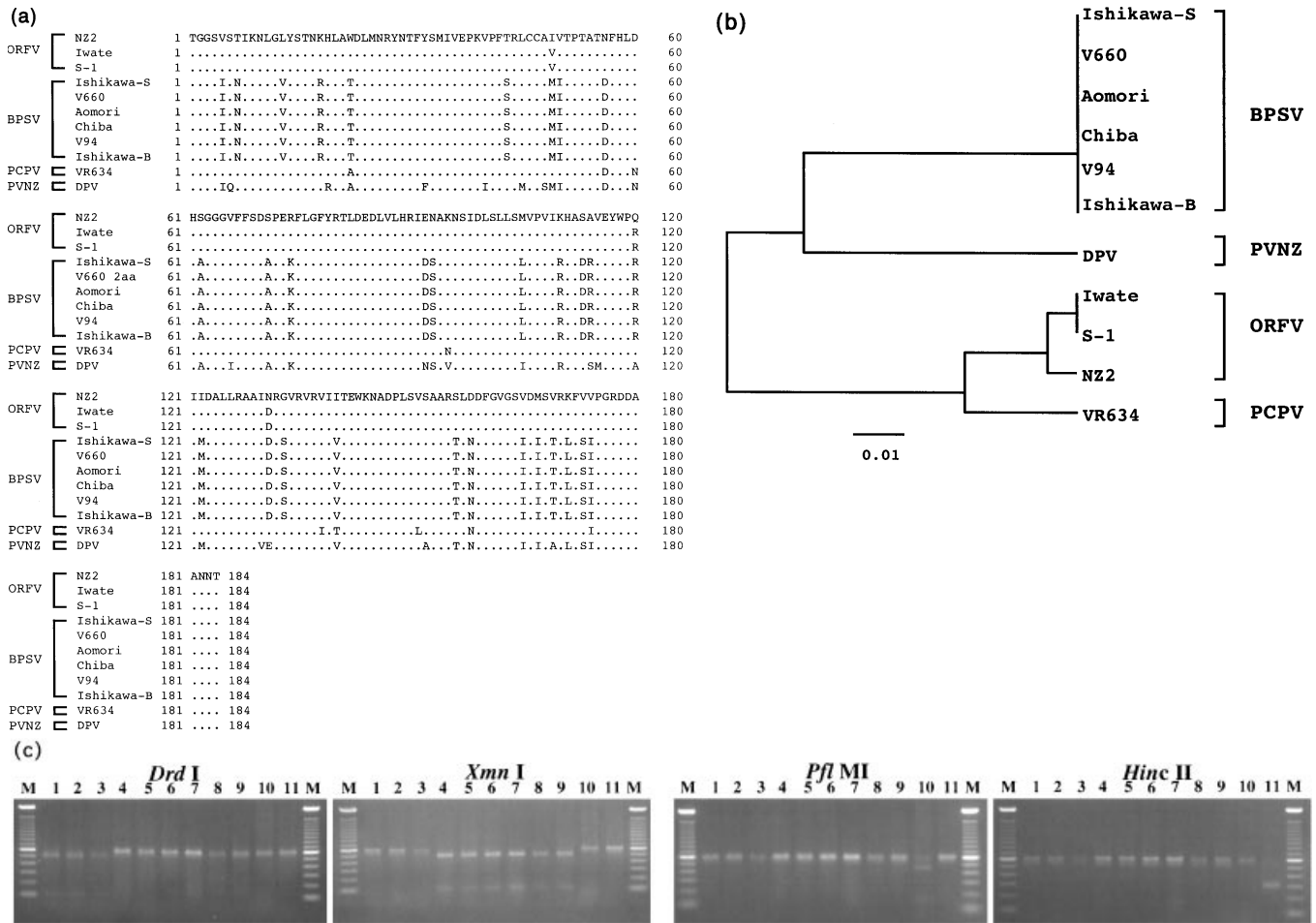


Fig. 2. (a) Alignment of the deduced amino acid sequences of partial envelope regions of parapoxviruses. Amino acids identical to ORFV strain N22 at given positions are represented by dots. (b) Phylogenetic tree based on amino acid sequences of partial envelope regions of parapoxviruses. The tree was constructed by the UPGMA method by using GENETYX-MAC version 9.0. (c) PCR-RFLP analysis of parapoxviruses. A 594 bp fragment from the envelope region was amplified from each strain. PCR products were digested with *DrdI*, *XmnI*, *PflMI* and *HincII*. PCR products from ORFVs, BPSVs, PCPV and PVNZ were respectively cut only by *DrdI* (500 and 94 bp), *XmnI* (496 and 98 bp), *PflMI* (443 and 151 bp) and *HincII* (306 and 288 bp). Lanes: M, 100 bp ladder; 1, N22; 2, Iwate; 3, S-1; 4, Ishikawa-S; 5, V660; 6, Aomori; 7, Chiba; 8, V94; 9, Ishikawa-B; 10, VR634; 11, DPV.

classification obtained by molecular analysis. Therefore, the methods for PCR, sequencing and RFLP reported here will be useful for genetic characterization and classification of parapoxviruses, especially when the virus can not be isolated.

We expected at first that the two strains from the wild Japanese serow might be classified into ORFV, because the Japanese serow belongs to the subfamily Caprinae and is more closely related taxonomically to sheep and goats than to cattle (Chikuni *et al.*, 1995). However, although strain S-1 was classified into ORFV as expected, Ishikawa-S belonged molecularly and antigenically to BPSV. Likewise, Southern blot analysis of S-1 and Ishikawa-S indicated that they were heterologous (Inoshima *et al.*, 2000b). These findings suggest that at least two different groups of parapoxviruses, ORFV and BPSV, are circulating and cause disease among Japanese

serows. Moreover, our data suggest that there are virus cycles between Japanese serows and domestic animals, such as from cattle or sheep to Japanese serows and vice versa. Further molecular analysis of parapoxviruses may reveal implications for pathogenesis and adaptation of the viruses in animals.

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References

- Bárcena, J., Lorenzo, M. M., Sánchez-Puig, J. M. & Blasco, R. (2000).** Sequence and analysis of a swinepox virus homologue of the vaccinia virus major envelope protein P37 (F13L). *Journal of General Virology* **81**, 1073–1085.
- Blake, N. W., Porter, C. D. & Archard, L. C. (1991).** Characterization of a molluscum contagiosum virus homolog of the vaccinia virus p37K major envelope antigen. *Journal of Virology* **65**, 3583–3589.
- Calvert, J. G., Ogawa, R., Yanagida, N. & Nazerian, K. (1992).** Identification and functional analysis of the fowlpox virus homolog of the vaccinia virus p37K major envelope antigen gene. *Virology* **191**, 783–792.
- Chikuni, K., Mori, Y., Tabata, T., Saito, M., Monma, M. & Kosugiyama, M. (1995).** Molecular phylogeny based on the κ -casein and cytochrome *b* sequences in the mammalian suborder Ruminantia. *Journal of Molecular Evolution* **41**, 859–866.
- Espósito, J. J., Baxby, D., Black, D. N., Dales, S., Darai, G., Dumbell, K. R., Granados, R. R., Joklik, W. K., McFadden, G., Moss, B., Moyer, R. W., Pickup, D. J., Robinson, A. J. & Tripathy, D. N. (1995).** Family Poxviridae. In *Virus Taxonomy. Sixth Report of the International Committee on Taxonomy of Viruses*, pp. 79–91. Edited by F. A. Murphy, C. M. Fauquet, D. H. L. Bishop, S. A. Ghabrial, A. W. Jarvis, G. P. Martelli, M. A. Mayo & M. D. Summers. Vienna & New York: Springer-Verlag.
- Fenner, F. (1996).** Poxviruses. In *Fields Virology*, 3rd edn, pp. 2673–2702. Edited by B. N. Fields, D. M. Knipe & P. M. Howley. Philadelphia: Lippincott–Raven.
- Friedman-Kien, A. E., Rowe, W. P. & Banfield, W. G. (1963).** Milker's nodules: isolation of a poxvirus from a human case. *Science* **140**, 1335–1336.
- Horner, G. W., Robinson, A. J., Hunter, R., Cox, B. T. & Smith, R. (1987).** Parapoxvirus infections in New Zealand farmed red deer (*Cervus elaphus*). *New Zealand Veterinary Journal* **35**, 41–45.
- Inoshima, Y., Shimizu, S., Minamoto, N., Hirai, K. & Sentsui, H. (1999).** Use of protein AG in an enzyme-linked immunosorbent assay for screening for antibodies against parapoxvirus in wild animals in Japan. *Clinical and Diagnostic Laboratory Immunology* **6**, 388–391.
- Inoshima, Y., Morooka, A., Murakami, K. & Sentsui, H. (2000a).** Simple preparation of parapoxvirus genome DNA for endonuclease analysis. *Microbiology and Immunology* **44**, 69–72.
- Inoshima, Y., Morooka, A. & Sentsui, H. (2000b).** Detection and diagnosis of parapoxvirus by the polymerase chain reaction. *Journal of Virological Methods* **84**, 201–208.
- 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. *Epidemiology and Infection* (in press).
- Kumagai, T., Shimizu, M., Ito, Y., Kanno, S., Nakagawa, M., Sato, K., Mukainakano, K. & Ohta, H. (1971).** Contagious papular dermatitis of sheep. In *Abstracts of the 71st Meeting of the Japanese Society of Veterinary Science*, p. 15. Tokyo, 1–3 April 1971 (in Japanese).
- Kumagai, T., Furuuchi, S. & Ito, Y. (1976).** Occurrence of bovine papular stomatitis. *National Institute of Animal Health Quarterly* **16**, 183–184.
- Kuroda, Y., Yoshida, M., Shibahara, T., Matsui, T., Nakane, T., Hara, H., Inoshima, Y. & Sentsui, H. (1999).** An epidemic of parapoxvirus infection among cattle: isolation and antibody survey. *Journal of Veterinary Medical Science* **61**, 749–753.
- Lard, S. L., Roehrig, J. T. & Pearson, L. D. (1991).** Differentiation of parapoxviruses by application of orf virus-specific monoclonal antibodies against cell surface proteins. *Veterinary Immunology and Immunopathology* **28**, 247–258.
- Mayr, A. & Büttner, M. (1990a).** Bovine papular stomatitis virus. In *Virus Infections of Ruminants*, pp. 23–28. Edited by Z. Dinter & B. Morein. Amsterdam: Elsevier.
- Mayr, A. & Büttner, M. (1990b).** Milker's node virus. In *Virus Infections of Ruminants*, pp. 29–32. Edited by Z. Dinter & B. Morein. Amsterdam: Elsevier.
- Mayr, A. & Büttner, M. (1990c).** Ecthyma (orf) virus. In *Virus Infections of Ruminants*, pp. 33–42. Edited by Z. Dinter & B. Morein. Amsterdam: Elsevier.
- Memar, O. & Tyring, S. K. (1995).** Cutaneous viral infections. *Journal of the American Academy of Dermatology* **33**, 279–287.
- Menna, A., Wittek, R., Bachmann, P. A., Mayr, A. & Wyler, R. (1979).** Physical characterization of a stomatitis papulosa virus genome: a cleavage map for the restriction endonucleases *Hind*III and *Eco*RI. *Archives of Virology* **59**, 145–156.
- Mercer, A., Fleming, S., Robinson, A., Nettleton, P. & Reid, H. (1997).** Molecular genetic analyses of parapoxviruses pathogenic for humans. *Archives of Virology Supplementum* **13**, 25–34.
- Moss, B. (1996).** Poxviridae: the viruses and their replication. In *Fields Virology*, 3rd edn, pp. 2637–2671. Edited by B. N. Fields, D. M. Knipe & P. M. Howley. Philadelphia: Lippincott–Raven.
- Ogino, H., Nakabayashi, D., Nabeya, M., Hoshi, K. & Okazawa, T. (1996).** Contagious papular dermatitis of Japanese serows in Niigata Prefecture. *Journal of Japanese Veterinary Medical Association* **49**, 615–618.
- Okada, H., Matsukawa, K. & Chihaya, Y. (1986).** Experimental transmission of contagious pustular dermatitis from a Japanese serow, *Capricornis crispus*, to a calf and goats. *Journal of Japanese Veterinary Medical Association* **39**, 578–581.
- Robinson, A. J. & Lyttle, D. J. (1992).** Parapoxviruses: their biology and potential as recombinant vaccines. In *Recombinant Poxviruses*, pp. 285–327. Edited by M. Binns & G. L. Smith. Boca Raton, FL: CRC Press.
- Robinson, A. J. & Mercer, A. A. (1995).** Parapoxvirus of red deer: evidence for its inclusion as a new member in the genus *Parapoxvirus*. *Virology* **208**, 812–815.
- Robinson, A. J., Ellis, G. & Balassu, T. (1982).** The genome of orf virus: restriction endonuclease analysis of viral DNA isolated from lesions of orf in sheep. *Archives of Virology* **71**, 43–55.
- Rosenbusch, R. F. & Reed, D. E. (1983).** Reaction of convalescent bovine antisera with strain-specific antigens of parapoxviruses. *American Journal of Veterinary Research* **44**, 875–878.
- Sentsui, H., Murakami, K., Inoshima, Y., Shibahara, T. & Yokomizo, Y. (1999).** Isolation of parapoxvirus from a cow treated with interferon- γ . *Veterinary Microbiology* **70**, 143–152.
- Sentsui, H., Inoshima, Y., Minami, A., Yamamoto, Y., Murakami, K. & Shimizu, S. (2000).** Survey on antibody against parapoxvirus among cattle in Japan. *Microbiology and Immunology* **44**, 73–76.
- Sullivan, J. T., Mercer, A. A., Fleming, S. B. & Robinson, A. J. (1994).** Identification and characterization of an orf virus homologue of the vaccinia virus gene encoding the major envelope antigen p37K. *Virology* **202**, 968–973.
- 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*). *Journal of Wildlife Disease* **29**, 384–389.
- Ueda, N. (1996).** Detection of orf virus cross-reacting antigens with other poxvirus antigens using monoclonal antibodies. *Journal of the Wakayama Medical Society* **47**, 443–451.

Wheeler, C. E. & Cawley, E. P. (1956). The microscopic appearance of ecthyma contagiosum (orf) in sheep, rabbits, and man. *American Journal of Pathology* **32**, 535–545.

Wittek, R., Herlyn, M., Schümperli, D., Bachmann, P. A., Mayr, A. & Wyler, R. (1980). Genetic and antigenic heterogeneity of different parapoxvirus strains. *Intervirology* **13**, 33–41.

Yata, S., Murakami, T., Ozawa, T. & Kitano, H. (1996). A case of parapoxvirus infection in wild Japanese serow (*Capricornis crispus*) in Ishikawa prefecture. *Japanese Journal of Zoo and Wildlife Medicine* **1**, 93–97.

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