ISOLATION AND ANTIBODY PREVALENCE OF A PARAPOXVIRUS IN WILD JAPANESE SEROWS (CAPRICORNIS CRISPUS)

Authors: Suzuki, Tomoko, Minamoto, Nobuyuki, Sugiyama, Makoto, Kinjo, Toshio, Suzuki, Yoshitaka, et al.

Source: Journal of Wildlife Diseases, 29(3) : 384-389

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-29.3.384

Journal of Wildlife Diseases, 29(3), 1993, pp. 384–389

ISOLATION AND ANTIBODY PREVALENCE OF A PARAPOXVIRUS IN WILD JAPANESE SEROWS (*CAPRICORNIS CRISPUS*)

Tomoko Suzuki,[1] Nobuyuki Minamoto,[1] Makoto Sugiyama,[1] Toshio Kinjo,[1] Yoshitaka Suzuki,[2] Makoto Sugimura,[2] and Yasuro Atoji[2]

[1] Department of Veterinary Public Health, Faculty of Agriculture, Gifu University, Yanagido, Gifu 501-11, Japan

[2] Department of Veterinary Anatomy, Faculty of Agriculture, Gifu University, Yanagido, Gifu 501-11, Japan

ABSTRACT: An epizootic suspected to be caused by parapoxviruses occurred in winter, 1984–85, among wild Japanese serows (*Capricornis crispus*) with nodular or papular lesions in Gifu Prefecture, Japan. Virus isolations were attempted on 30 animals using bovine fetal testicle cell cultures. Viral agents growing with cytopathic effects were isolated from six animals. The agents were identified as parapoxvirus from electron microscopic findings and physico-chemical characteristics.

Antibody prevalence to the isolated agent, named S-1, was examined by enzyme-linked immunosorbent assay. No antibodies were detected among 153 sera obtained during two winters 1981–82 and 1982–83, but one of 189 sera collected in the winter of 1983–84 was positive. In contrast, 75 (32%) of 237 sera had antibodies to S-1 in 1984–85, when the disease was first detected. Antibody prevalences of 1984–85 were highest (39%) in December 1984, the first month of animal capture, and declined gradually to 20% in March 1985.

Key words: Parapoxvirus, Japanese serow, *Capricornis crispus*, virus isolation, serological survey, enzyme-linked immunosorbent assay (ELISA), epizootiology.

INTRODUCTION

The Japanese serow (*Capricornis crispus*), the only wild ruminant in Japan, is protected. But in recent years the Agency of Cultural Affairs of the Japanese Government has permitted limited killing of serows in the winter season (December to March) to prevent their damage to forests. In Gifu Prefecture, the central part of mainland Japan, approximately 400 serows per year were killed from 1979 to 1984, and all carcasses were evaluated at Gifu University, Gifu, Japan.

In the course of these inspections, we first encountered an epizootic of a disease suspected to be a parapoxvirus infection in the winter of 1984–85. Nodular or papular lesions were found primarily in the naso-oral, external genital, udder, and auricular parts. Such lesions were present on 155 (39%) of 402 serows studied. Based on histopathologic and electron microscopic findings, we proposed that the disease might be caused by a parapoxvirus (Suzuki et al., 1986).

To test this hypothesis, virus isolations were attempted from the lesions. Moreover, antibody prevalence to the isolated agent was determined among serows killed from December through March, each year from 1981 to 1985.

MATERIALS AND METHODS

Wild Japanese serows were killed in forested areas (36°05′N, 137°20′E) of Gifu Prefecture from December 1984 through March 1985. Thirty animals with severe lesions were evaluated. Nodular or papular lesions from each animal were obtained and a 10% suspension was made using a homogenizer (Teraoka, Tokyo, Japan) in Hanks' solution as described by Minamoto et al. (1988). After centrifugation at 1,000 × g for 10 min, supernates were stored at −80 C for subsequent use in isolation procedures.

For virus isolation, primary or secondary bovine fetal testicle (BFT) monolayers were used as growth media. For primary cultures the trypsinized cells were suspended at 5.0×10^5 cells/ml in Eagle's minimum essential medium (MEM) (Nissui Seiyaku, Tokyo, Japan) supplemented with 10% calf serum and 0.295% tryptose phosphate broth (TPB) and incubated stationary at 37 C. For secondary cultures, BFT cells were suspended at 7.5×10^4/ml in the Eagle's MEM with 5% calf serum and 0.148% TPB. Penicillin (Banyu Seiyaku, Tokyo, Japan) (100 units/ml), streptomycin (Meiji Seika, Tokyo) (100 μg/ml) and amphotericin B (Sankyo Seiyaku, Tokyo) (25 μg/ml) were added to the growth media for both primary and secondary cultures.

After washing three times with Hanks' solu-

tion, primary BFT cells grown in 13 × 100 mm tissue culture tubes were inoculated with 0.1 ml/tube of lesion material for isolation and adsorbed for 1 hr at 37 C. Then, BFT cells were washed once with Hanks' solution; 0.5 ml maintenance medium (growth medium for secondary cultures that is calf-serum free) was added, and the BFT cells were incubated at 37 C for 14 days. When the cytopathic effect (CPE) involved more than 50% of the cell monolayer, the culture fluids were harvested and centrifuged at 1,000 × g for 10 min. The supernatant fluid was frozen at −80 C. When no CPE appeared for 14 days, the cells and fluids were harvested and centrifuged in the same manner. Each supernatant was subinoculated into BFT cells for a second passage. Three serial blind passages were done before a sample was considered negative. Infectivity titrations of parapoxviruses were conducted as follows. Each tenfold serial dilution of virus materials tested was added to four wells of a microplate with 0.025 ml/well; 0.1 ml of 7.5×10^4/ml BFT cells then was added. After the virus-cell mixtures were shaken gently, plates were incubated for 14 days at 37 C in an incubator with 5% carbon dioxide. The mean tissue culture infective dose ($TCID_{50}$) was calculated by the method of Reed and Muench (1938).

Coverslip cultures of BFT cells in which CPE appeared were fixed in Bouin's solution (15 parts saturated picric acid, five parts 40% formaldehyde, and one part glacial acetic acid). The cultures were stained with hematoxylin and eosin (H&E), and examined under light microscopy for cytoplasmic inclusions. Fluids obtained from cultures of BFT cells with CPE were clarified by centrifugation at 7,000 × g for 20 min; the supernatant fluids were centrifuged at 140,000 × g for 2 hr. The resulting pellets were dissolved in NTE (NaCl-tris-EDTA) buffered solution (Sokol et al., 1968) to 1/100 of the original volume of culture fluids. The concentrated materials were negatively stained with 2% phosphotungstic acid (Wako Junyaku, Tokyo, Japan) (Minamoto et al., 1978). Virus material prepared directly from 10% suspensions of papular or nodular lesions also was concentrated and stained in the same manner. Further, ultra-thin sections of BFT cells showing CPE were prepared as described by Minamoto et al. (1978). The stained preparations were examined by a Hitachi H-800 electron microscope (Hitachi, Tokyo).

The effect of the anti-DNA synthetic drug, 5-iodo-2′-deoxyuridine (IUdR) (Sigma Chemical Company, St. Louis, Missouri, USA) at 50 μg/ml, for virus growth was determined by the method of Minamoto et al. (1988). Simian virus 40 (SV40) and a rotavirus were used for comparison. Sensitivities to lipid solvents were tested using chloroform and ether as described by Minamoto et al. (1978). As controls, akabane virus and a rotavirus were used.

Serows were killed between December 1981 to March 1982 (n = 50), December 1982 to March 1983 (n = 103), December 1983 to March 1984 (n = 189), and December 1984 to March 1985 (n = 237). Their sera were evaluated for parapoxvirus antibodies with an enzyme-linked immunosorbent assay (ELISA) using the method of Kavaklova et al. (1984). The viral antigen was prepared from infected BFT cells and purified antigen was adjusted to a final protein concentration of 4 μg/ml in 0.05 M carbonate buffered solution (Minamoto et al., 1988). The antigen (0.05 ml) was delivered into wells of ELISA microplates (Nunc, Kamstrup, Denmark) and incubated overnight at 4 C. The microplates were irradiated with ultraviolet light from a 15-watt germicidal lamp for 5 min, at a distance of 10 cm; excess antigen was discarded. Phosphate buffered saline containing 0.05% of Tween 20 (PBS-T) and 1% of calf serum (0.1 ml/well) was added and incubated at 37 C for 2 hr. After washing once, 0.05 ml of diluted (1:4) test serum was added and incubated at 37 C for 1 hr. Wells were rinsed five times with PBS-T and then 0.05 ml of diluted (1:1,000) peroxidase conjugated protein A (E-Y Laboratories, San Mateo, California, USA) was added for 1 hr at 37 C. After washing five times as before, 0.05 ml of 0.05 M citrate phosphate buffered solution with 0.05 M o-phenylenediamine (Sigma) and 0.01% hydrogen peroxide was added to each well and incubated 30 min in the dark. The enzyme reaction was terminated by addition of 0.05 ml of 4 N sulfuric acid (H_2SO_4)/well. Each optical density (OD) value was obtained at 490 nm. For determining the optimum condition and cut-off value in the ELISA, one diseased serow serum having anti-S-1 neutralizing antibody titer (≥1:16) was used as a positive antibody control and 50 sera of apparently healthy serows captured in 1981 were used as negative antibody controls. The OD value was 0.65 in the positive control and the mean OD value was 0.02 (±0.02, standard deviation) in the negative controls. Determination of the cut-off value at ≥0.13 was based on the mean plus five standard deviations of negative controls.

RESULTS

Cytopathic effects were observed in inoculated BFT cell cultures with six of 30 lesions tested. Two of the six appeared at 6 and 7 days, respectively, after inoculation in first passage and the other four at

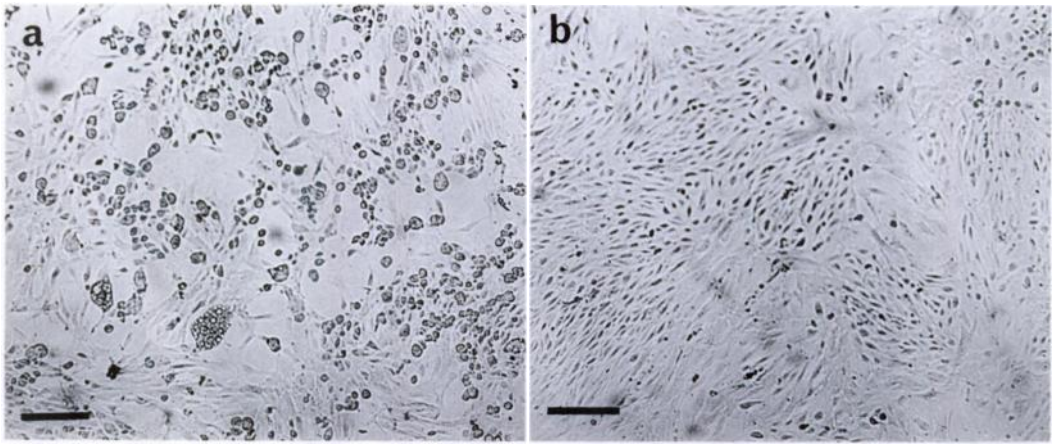

FIGURE 1. (a) Cytopathic effect in bovine fetal testicle cells seven days after inoculation with S-1 parapoxvirus strain and (b) uninoculated control. Unstained. Bar = 1 mm.

4 to 7 days in the second passage. The CPE in BFT cells consistently appeared as foci of rounded cells and developed slowly; eventually, infected cells detached (Fig. 1). The virus isolated first was designated S-1 strain and was subsequently used for all characterization studies.

Viruses were isolated from four juvenile serows (<1-yr-old), one 2-yr-old, and one >10-yr-old. Three were males and three were females.

In H&E stained preparations of BFT cells inoculated with the S-1 strain, eosinophilic cytoplasmic inclusions surrounded by a halo were observed (Fig. 2). With the electron microscope many elliptical viral particles with tubular structure were observed in cytoplasm of the infected BFT cells. Negatively staining particles from six tissue culture fluids showing CPE and from suspensions of lesions from which the S-1 strain recovered, had characteristics typical of parapoxviruses. They had a spiral "ball of wool" appearance with thick, tubular, parallel fibrils which crossed the longitudinal axis of the particles. The average size of viral particles from these infected cells was 283 × 163 nm (Fig. 3).

Infectivity of the S-1 strain was inhibited by 50 μg/ml IUdR, suggesting the

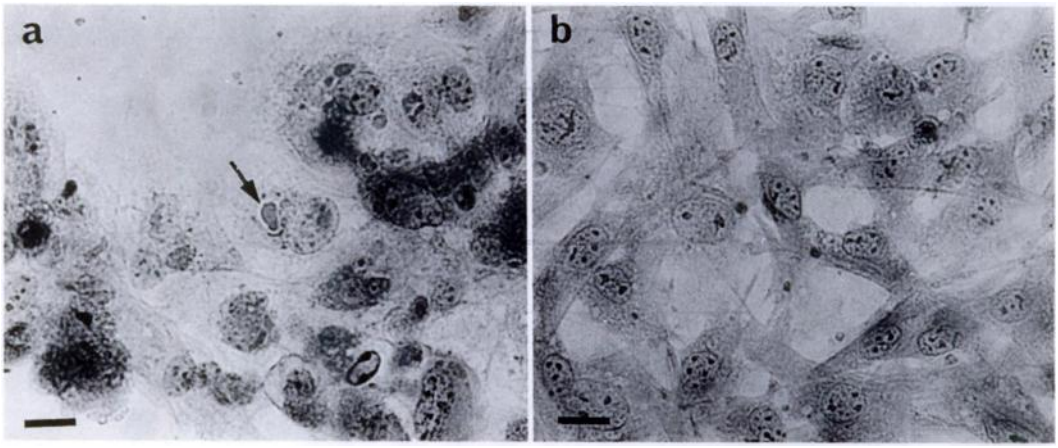

FIGURE 2. (a) Cytoplasmic inclusion (arrow) in bovine fetal testicle cells inoculated with S-1 parapoxvirus strain and uninoculated cell control. H&E. Bar = 200 μm.

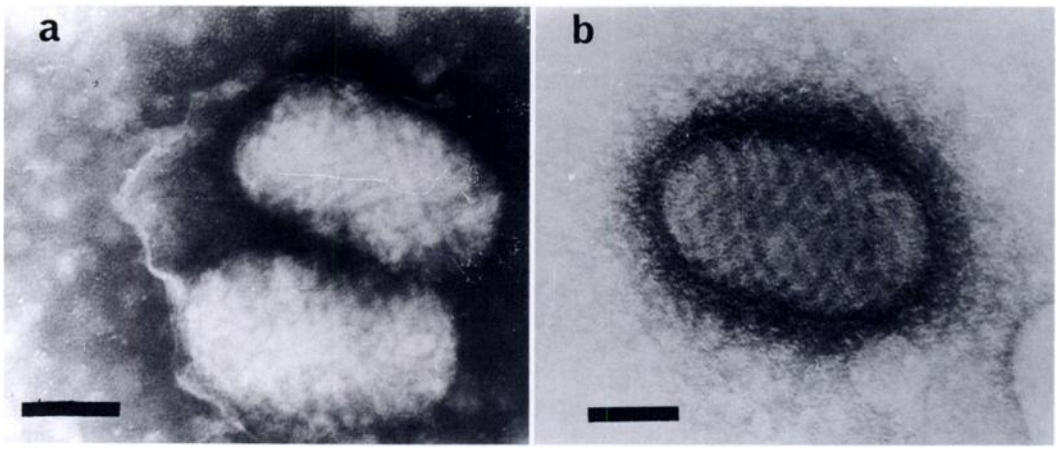

FIGURE 3. Negatively stained electron micrographs of (a) viral particles from the fluid of bovine fetal testicle cells infected with S-1 strain and (b) particle from a 10% suspension of a papular lesion from which S-1 parapoxvirus strain was isolated. Bar = 100 nm.

virus contained DNA (Table 1). The S-1 strain also was sensitive to chloroform and partially resistant to ether (Table 2). Based on these results, we believe that the S-1 virus strain is a parapoxvirus.

Antibody prevalence was 0 of 50 serows in the winter of 1981–82, 0 of 103 in 1982–83, and 1 of 189 in 1983–84. In contrast to previous winters, 75 (32%) of 237 serows were seropositive during the winter of 1984–85, when overt disease first was detected. Antibody prevalence in the winter 1984–85 was the highest in December 1984 (27 of 69 serows, 39% prevalence) but declined gradually to 27 (33%) of 82 serows in January 1985, 17 (26%) of 66 in February, and 4 (20%) of 20 in March. No correlation of antibody prevalence to sex, age, and area captured was observed.

DISCUSSION

For virus isolation, we used BFT cells, but only two of 30 lesions produced CPE on the first BFT cell cultures and four other lesions showed CPE after the second blind passage. Judging from our results and those of others (Nagington, 1968; Moreno-Lopez and Lif, 1979), it is difficult to isolate parapoxvirus by cell cultures.

Viruses isolated in BFT cells were morphologically similar to those seen in partially purified preparations from lesions of serows and had the morphologic characteristics of parapoxviruses described by other investigators (Rossi et al., 1977; Moreno-Lopez and Lif, 1979; Okada et al., 1984a). The agent was also confirmed as a parapoxvirus by physico-chemical characteristics (Rossi et al., 1977; Moreno-Lopez and Lif, 1979; Abraham et al., 1985). To determine to which parapoxvirus S-1 was most closely related, we attempted cross-neutralization tests between S-1, Orf virus and bovine papular stomatitis virus (data not shown). But results were not conclusive because neutralizing antibody titers of corresponding sera were low. Neutralizing antibody to parapoxvirus is difficult to demonstrate (Rossi et al., 1977; Moreno-Lopez and Lif, 1979), and there are as yet no established serological guide-

TABLE 1. Effect of 5-iodo-2′-deoxyuridine on infectivity of S-1 strain in bovine fetal testicle cells.

Virus	Without IUdR[a]	With 50 μg/ml IUdR
S-1	6.3[b]	<1.0
Rotavirus	7.8	7.8
SV40	6.6	4.1

[a] IUdR is 5-iodo-2′-deoxyuridine.
[b] Log tissue culture infective dose/ml ($TCID_{50}$/ml).

TABLE 2. Effect of chloroform and ether on infectivity of S-1 strain in bovine fetal testicle cells.

Virus	Chloroform (10%)	Ether (20%)	Untreated control
S-1	<1.0[a]	5.7	7.2
Akabane	<1.0	<1.0	6.9
Rotavirus	8.6	8.6	8.6

[a] Log tissue culture infective dose/ml ($TCID_{50}$/ml).

lines for differentiating parapoxvirus strains (Wittek et al., 1980; Rosenbusch and Reed, 1983; Lard et al., 1991). However, Gassmann et al. (1985) showed that parapoxvirus strains can be classified by endonuclease analysis. Thus, further classification of the parapoxvirus from the serows is needed.

In Japan, sporadic epizootics of parapoxvirus infection in cattle have been reported since 1969 in northern parts (40°40'N, 140°55'E) of the mainland (Kumagai et al., 1976). In the same region, several epizootics of the disease occurred in wild Japanese serows during 1976 to 1981 (Okada et al., 1984a). However, no epizootics of parapoxvirus infection in serows or cattle in this region have been reported since. The cases reported in the present study occurred 3 yr later, approximately 800 km to the southwest. Because no viruses were isolated from serows in the north, we could not compare these diseases etiologically; but judging by the similarity in clinical signs and histopathologic findings (Okada et al., 1984a, b; Suzuki et al., 1986), they might have been caused by the same virus. The antibody prevalence in the winter of 1984–85 coincides with our previous published report on the occurrence and severity of the lesions, in which the prevalence of the disease was 46% in December, 43% in January, 23% in February and 31% in March; macroscopic lesions also were severe from December to January (Suzuki et al., 1986). Based on the serologic results, we propose that the disease began in the serow population early in 1984 and subsequently became widespread.

It is well known that parapoxviruses elicit few or no detectable antibodies (Rossi et al., 1977; Moreno-Lopez and Lif, 1979; Smith et al., 1991). Therefore, the disease might be more prevalent among serows than the antibody prevalences indicate. On the other hand, to heighten sensitivity, test procedures of ELISA should be improved and alternative serologic tests should be considered. Since the studies on the management and preservation of Japanese serows were finished by March 1985, we could not obtain samples in the winter of 1985 and thereafter. According to hunter observations, the disease ceased after the widespread epizootics in 1984 and appeared to have no direct effect on the decrease in the serow population.

As parapoxviruses maintain their infectivity in natural environments for long periods (McKeever and Reid, 1986) and also infect humans (Groves et al., 1991; Smith et al., 1991), close surveillance in these areas will be necessary.

ACKNOWLEDGMENTS

We thank many students for assistance with blood-sampling from Japanese serows. This study was supported in part by Grant-in-Aids for Cooperative Research (A) No. 58362001 (chief researcher: M. Sugimura) and for Developmental Scientific Research No. 03506001 (chief researcher: K. Hirai) from the Ministry of Education, Science and Culture of Japan.

LITERATURE CITED

ABRAHAM, A., M. DAVIDSON, AND M. SCHCLAREK. 1985. First isolation of bovine stomatitis papulosa virus in Israel. The Veterinary Record 116: 379.

GASSMANN, U., R. WYLER, AND R. WITTER. 1985. Analysis of parapoxvirus genomes. Archives of Virology 83: 17–31.

GROVES, R. W., E. WILSON-JONES, AND D. M. MACDONALD. 1991. Human orf and milkers' nodule: A clinicopathologic study. Journal of the American Academy of Dermatology 25: 706–711.

KAVAKLOVA, L., M. ESKENAZY, T. GANCHEVA, AND V. VACHEVA. 1984. Enzyme linked immunosorbent assay for quantification of rabies antibodies in human sera. Acta Virology 28: 422–427.

KUMAGAI, T., S. FURUUCHI, AND Y. ITO. 1976. Occurrence of bovines papular stomatitis. National

Institute of Animal Health Quarterly 16: 183–184.

LARD, S. L., J. T. ROEHRIG, AND L. D. PEARSON. 1991. Differentiation of parapoxviruses by application of orf virus-specific monoclonal antibodies against cell surface proteins. Veterinary Immunology and Immunopathology 28: 247–258.

MCKEEVER, D. J., AND H. W. REID. 1986. Survival of orf virus under British winter conditions. The Veterinary Record 118: 613–614.

MINAMOTO, N., Y. TAMURA, N. HIRAYAMA, H. SUZUKI, K. KURATA, H. SAZAWA, AND I. YAMAMOTO. 1978. An electron microscopic study of the surface structures and hemadsorption on chick embryo cells infected with rabies virus. Archives of Virology 58: 95–109.

———, K. OKI, T. TOMITA, T. KINJO, AND Y. SUZUKI. 1988. Isolation and characterization of rotavirus from feral pigeon in mammalian cell cultures. Epidemiology and Infection 100: 481–492.

MORENO-LOPEZ, J., AND I. LIF. 1979. A parapoxvirus isolated from nasal secretion of a calf with respiratory disease. Veterinary Microbiology 4: 85–88.

NAGINGTON, J. 1968. The growth of paravaccinia viruses in tissue culture. The Veterinary Record 82: 477–482.

OKADA, H. M., K. OKADA, S. NUMAKUNAI, AND K. OSHIMA. 1984a. Histopathologic studies on mucosal and cutaneous lesions in contagious papular dermatitis of Japanese serow (*Capricornis crispus*). Japanese Journal of Veterinary Science 46: 257–264.

———, ———, ———, AND ———. 1984b. Electron microscopy on mucosal and cutaneous lesions in contagious papular dermatitis of Japanese serows (*Capricornis crispus*). Japanese Journal of Veterinary Science 46: 297–302.

REED, L. J., AND H. MUENCH. 1938. A simple method of estimating fifty per cent end-points. American Journal of Hygiene 27: 493–497.

ROSENBUSCH, R. F., AND D. E. REED. 1983. Reaction of convalescent bovine antisera with strain-specific antigens of parapoxviruses. American Journal of Veterinary Research 44: 875–878.

ROSSI, C. R., G. K. KIESEL, AND M. H. JONG. 1977. A paravaccinia virus isolated from cattle. Cornell Veterinarian 57: 72–80.

SMITH, K. J., H. G. SKELTON, W. D. JAMES, AND G. P. LUPTON. 1991. Parapoxvirus infections acquired after exposure to wildlife. Archives of Dermatology 127: 79–82.

SOKOL, F., E. KUWART, T. J. WIKTOR, K. HUMMLER, AND H. KOPROWSKI. 1968. Purification of rabies virus growth in tissue culture. Journal of Virology 2: 836–849.

SUZUKI, Y., M. SUGIMURA, Y. ATOJI, N. MINAMOTO, AND T. KINJO. 1986. Widespread of parapoxvirus infection in wild Japanese serows, *Capricornis crispus*. Japanese Journal of Veterinary Science 48: 1279–1282.

WITTEK, R., M. HERLYN, D. SCHUMPERLI, AND P. A. BACHMANN. 1980. Genetic and antigenic heterogeneity of different parapoxvirus strains. Intervirology 13: 33–41.

Received for publication 21 August 1992.