Use of Protein AG in an Enzyme-Linked Immunosorbent Assay for Screening for Antibodies against Parapoxvirus in Wild Animals in Japan

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Using protein AG in an enzyme-linked immunosorbent assay (ELISA), we tried to detect antibodies against parapoxvirus in 9 species of wild animals in Japan: the Japanese badger (Meles meles anakuma), Japanese black bear (Ursus thibetanus japonicus), Japanese deer (Cervus nippon centralis), Japanese monkey (Macaca fasciata), Japanese raccoon dog (Nyctereutes procyonoides viverrinus), Japanese serow (Capricornis crispus), Japanese wild boar (Sus scrofa leucomystax), masked palm civet (Paguma larvata), and nutria (Myocastor coypus). A total of 272 serum samples were collected over the period from 1984 to 1995 and were tested by the protein AG-ELISA, the agar gel immunodiffusion test, and an indirect immunofluorescence assay. The protein AG-ELISA was effective in a serological survey for parapoxvirus in wild animals, and antibodies were detected only in Japanese serows. A total of 24 of 66 (36.4%) Japanese serows reacted positively, and they were found in almost all prefectures in all years tested. These results suggest that epizootic cycles of parapoxvirus exist widely in Japanese serows and that they could be reservoirs for the virus in the field in Japan. Moreover, it is probable that they might carry the virus to domestic animals such as cattle, sheep, and goats.

The genus Parapoxvirus includes bovine papular stomatitis virus and pseudowpox virus in cattle and orf virus in sheep and goats (15). The parapoxviruses cause a disease characterized by a contagious papular dermatitis around the mouth, teats, or skin of infected animals. The members of the Parapoxvirus genus are immunologically closely related and exhibit serological cross-reactivity (11, 16, 21, 22, 28). The viruses occasionally infect humans after close contact of humans with the skin lesions of infected animals or the handling of virus-contaminated materials, and the infections are therefore known as zoonoses (5, 12, 13, 19, 23, 25).

Parapoxvirus infections have also been described in other animals such as camels, seals, reindeer, musk ox, and squirrels (4, 19). Recently, a new parapoxvirus was isolated from red deer in New Zealand (6, 20). Thus, parapoxvirus infection may exist among wild animals in Japan, especially Japanese deer. However, data that support this speculation are available only for Japanese serows (17, 18, 26, 27). Since the foraging ranges of wild animals overlap those of domestic animals in pastures in certain areas, there is a possibility that wild animals infected with parapoxvirus are a factor in the spread of parapoxvirus infection among domestic animals.

In the study described here, we examined the utility of protein AG for use in a serological survey for evidence of parapoxvirus infection in nine species of wild animals in Japan and showed the prevalence of antibodies against parapoxvirus in the animals.

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MATERIALS AND METHODS

Serum samples. A total of 272 serum samples were collected over the period from 1984 to 1995 from nine species of wild animals in Japan, as shown in Table 1 and Fig. 1. They included the Japanese badger (Meles meles anakuma), Japanese black bear (Ursus thibetanus japonicus), Japanese deer (Cervus nippon centralis), Japanese monkey (Macaca fasciata), Japanese raccoon dog (Nyctereutes procyonoides viverrinus), Japanese serow (Capricornis crispus), Japanese wild boar (Sus scrofa leucomystax), masked palm civet (Paguma larvata), and nutria (Myocastor coypus). A total of 272 serum samples were collected over the period from 1984 to 1995. These results suggest that epizootic cycles of parapoxvirus exist widely in Japanese serows and that they could be reservoirs for the virus in the field in Japan. Moreover, it is probable that they might carry the virus to domestic animals such as cattle, sheep, and goats.
TABLE 1. Wild animals tested for antibodies against parapoxvirus

| Animal                          | Genus and species          | No. of serum samples tested | Period of serum collection | Prefectures where sera were collected |
|---------------------------------|---------------------------|----------------------------|----------------------------|---------------------------------------|
| Japanese badger                 | Meles meles anakuma       | 2                          | 1992                       | Gifu                                  |
| Japanese black bear             | Ursus thibetanus japonicus| 30                         | 1991–1993                  | Gifu, Shiga                           |
| Japanese deer                   | Cervus nippon centralis   | 55                         | 1991–1993                  | Aomori, Iwate, Miyagi, Hyogo          |
| Japanese monkey                 | Macaca fuscata            | 30                         | 1991–1992                  | Gifu                                  |
| Japanese raccoon dog            | Nyctereutes procyonoides viverinus | 24                      | 1991–1992                  | Gifu, Mic                             |
| Japanese serow                  | Capricornis crispus       | 66                         | 1984–1995                  | Yamagata, Tochigi, Kanagawa, Gifu     |
| Japanese wild boar              | Sus scrofa leucomystax    | 30                         | 1991–1992                  | Gifu, Shiga, Mic, Hyogo               |
| Masked palm civet               | Paguma larvata            | 5                          | 1991–1992                  | Gifu                                  |
| Nutria                          | Myocastor cooperi         | 30                         | 1991–1992                  | Gifu                                  |

RESULTS

Protein binding capacity. Since no data on the binding capacities of proteins A and G to immunoglobulins of wild animals in Japan were available, serum samples from all species were tested with proteins A, G, and AG. Five serum samples selected from each animal at random were coated onto a microplate, and the binding capacity was tested by ELISA as described above. Sera from most species reacted with both proteins A and G, whereas those from the Japanese badger, Japanese black bear, and masked palm civet reacted only with protein A. Sera from the Japanese serow reacted strongly with protein A (Table 2). Protein AG had a broad binding ability and bound to sera from nine species, and it was therefore used for further serological experiments.

Detection of antibody. A total of 272 serum samples were tested for the prevalence of antibody to parapoxvirus. In the protein AG-ELISA, the cutoff value was determined on the basis of the bimodal distribution of the antibody titer for each species. Most OD values for seronegative samples from wild animals were almost the same as those for the seronegative control samples from cattle. We designated as positive serum samples with OD values more than threefold that for the negative control.

Antibodies against parapoxvirus were detected only in Japanese serows by the protein AG-ELISA (Fig. 2 and Table 3). The seroprevalence of antibodies against parapoxvirus among Japanese serows was 24 of 66 (36.4%), and seropositive serows were found in almost all prefectures in all years tested. The OD value for seropositivity ranged from 0.10 to 0.55 (Fig. 2). Seropositive samples were also determined to be positive by both the AGID test and IFA, in which a precipitation line and specific fluorescence were observed, respectively (data not shown). For some samples from other species and for one...
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ric protein AG can strongly bind to the immunoglobulins of

many mammalian species but not to those of birds and reptiles

(9), it is a powerful tool for use in immunological studies (1, 2).

It is especially very useful for serological surveys of infectious

agents in wild animals because antisera against immunoglobu-

ins of wild animals are usually not commercially available. Among the sera from the nine species examined in this study, sera from six species reacted with both proteins A and G, whereas sera from three species reacted with only one protein. The protein AG-ELISA developed in this study could detect immunoglobulins against parapoxivirus in all species and might be applicable to serological surveys of the virus in other wild animals.

Antibodies against parapoxvirus were detected only in Japa-

nese serows and were not detected in the other species tested. Parapoxvirus infections in Japanese serows were first reported in 1976 in Akita Prefecture (9a) and began to be observed in other areas, such as Aomori Prefecture in 1978 (27a) and Iwate, Yamagata, Fukushima, and Miyagi Prefectures from 1979 to 1982 (8, 18) but remained limited in the prefectures in the northern part of Japan (Fig. 1). In Gifu Prefecture, which is located in central part of Japan, a previous study demonstrated that although no antibodies were detected until the winter of 1982-1983, antibodies were detected from 1 of 189 serum samples in the winter of 1983-1984 and the disease occurred in the winter of 1984-1985 in Japanese serows (26). The disease is now spreading to other areas, such as Tokyo and Ishikawa (29, 31) (Fig. 1). These observations and our results suggest that the infections spread from northern Japan to central Japan and epizootic cycles of parapoxvirus infection are widely prevalent in Japanese serows.

A previous report showed that 37% of cattle in Aomori Pre-

fecture in 1980 were seropositive for parapoxvirus (27a). How-

ever, our survey in 1998 revealed that 100% of cattle over 5

years old in Chiba Prefecture and the prefectures near Chiba

Prefecture were positive for parapoxvirus (10). The increase in

the rate of seropositivity among cattle may be associated with

the spread of the disease in Japanese serows and with the

increase in the rate of cattle transfer. The roles of some wild

animals as reservoirs and/or amplifiers of viruses such as Af-

rican swine fever and rabies viruses are reasonably well known.

Japanese serows could be reservoirs for the virus in the field in

Japan and might carry parapoxvirus to domestic animals such

as cattle, sheep, and goats. It is also likely that there are virus

cycles among Japanese serows and domestic animals.

Recently, a new parapoxvirus was isolated from red deer in

New Zealand (6, 20). Clinical symptoms in red deer were ob-

served at some geographically isolated farms. The isolated vi-

rus was genetically distinguishable from other parapoxviruses

(6, 20). We first suspected the presence of antibodies in Jap-

anese deer, but no antibodies were detected. However, there is

a possibility that the virus could be introduced into Japanese

der by foreign deer that carry it. In 1997, contagious pustular

dermatitis in captive Japanese livestock deer was added to the

list of infectious diseases that we must monitor, according to

| Animal                  | Protein AG | Protein A | Protein G |
|-------------------------|------------|-----------|-----------|
| Japanese monkey         | 1.41 (0.03)| 1.68 (0.07)| 1.51 (0.05) |
| Japanese deer           | 1.38 (0.05)| 1.28 (0.27)| 1.40 (0.03) |
| Japanese badger         | 1.37 (0.00)| 1.50 (0.03)| 0.02 (0.01) |
| Japanese wild boar      | 1.33 (0.11)| 1.41 (0.32)| 1.23 (0.18) |
| Nutria                  | 1.25 (0.15)| 1.55 (0.19)| 1.24 (0.13) |
| Japanese raccoon dog    | 1.19 (0.23)| 1.04 (0.37)| 0.56 (0.27) |
| Masked palm civet       | 1.11 (0.51)| 1.09 (0.55)| 0.02 (0.01) |
| Japanese black bear     | 0.98 (0.51)| 1.09 (0.67)| 0.09 (0.08) |
| Japanese serow          | 0.75 (0.45)| 0.15 (0.18)| 1.08 (0.33) |

* Five serum samples from each animal except Japanese badgers were selected at random and were tested with proteins AG, A, and G. Two serum samples from Japanese badgers were tested.

### Table 3: Geographic and temporal distribution of seropositive Japanese serows

| Year       | No. of seropositive serows/total no. of serows tested (%) | in the following prefecture: |
|------------|----------------------------------------------------------|-----------------------------|
| 1984-1985* | 14/30 (46.7)                                              | Gifu | Kanagawa | Tochigi | Yamagata |
| 1989       | 0/1 (0.0)                                                 |     |          |        |
| 1991       | 1/2 (50.0)                                                | 2/3 (66.7)                  |     |        |
| 1992       | 0/5 (0.0)                                                 | 1/6 (16.7)                  |     |        |
| 1993       | 0/3 (0.0)                                                 | 3/5 (60.0)                  |     |        |
| 1994       | 2/5 (40.0)                                                |     |          | 1/6 (16.7) |
| 1995       | 1/6 (16.7)                                                |     |          |        |

* Winter season.

![FIG. 2. OD values for sera from Japanese serows in the protein AG-ELISA.](image)
the Animal Infectious Disease Control Law in Japan. Since there are no reports of clinical observations or infections at present, we can say that Japanese deer appear to have been free of this disease, at least until 1993.

Parapoxvirus infection was seen in many animals. The members of the genus Parapoxivirus are closely related, and there are no established serological distinctions (11, 16, 21, 22, 28). The relationship between the virus that infects wild animals and other parapoxviruses is still unclear. Restriction endonuclease analysis of viral DNA and DNA-DNA hybridization analyses are thought to be useful methods for the classification of parapoxviruses (14, 19). Thus, isolation of virus from Japanese serows and endonuclease analysis are required to clarify the relationship between parapoxvirus and other parapoxviruses in serows.

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REFERENCES

1. Etraisson, M., R. Andersson, A. Olsson, H. Wigzell, and M. Uhlen. 1989. Differential IgG-binding characteristics of staphylococcal protein A, streptococcal protein G, and a chimeric protein AG. J. Immunol. 142:575–581.
2. Etraisson, M., A. Olsson, E. Palmenrantz, K. Wiberg, M. Inganäs, B. Gass, M. Lindberg, and M. Uhlen. 1988. Chimeric IgG-binding receptors engineered from staphylococcal protein A and streptococcal protein G. J. Biol. Chem. 263:4323–4327.
3. Esposito, J. J., J. F. Objieski, and J. H. Nakano. 1978. Orthopoxvirus DNA: strain differentiation by electrophoresis of restriction endonuclease fragmented virion DNA. Virology 89:53–66.
4. Falk, E. S. 1978. Parapoxvirus infections of reindeer and musk ox associated with unusual human infections. Br. J. Dermatol. 99:647–654.
5. Fenner, F. 1996. Poxviruses, p. 2673–2702. In B. N. Fields, D. M. Knipe, and P. M. Howley (ed.), Fields virology, 3rd ed. Lippincott-Raven Publishers, Philadelphia, Pa.
6. Horner, G. W., A. J. Robinson, R. Hunter, B. T. Cox, and R. Smith. 1987. Parapoxvirus infections in New Zealand farmed red deer (Cervus elaphus). N. Z. Vet. J. 35:41–45.
7. Imada, T., T. Tsuboi, N. Takahashi, T. Hamaoka, M. Haritani, T. Miyamoto, and H. Murata. 1996. Serological survey of 8 bovine viral pathogens in sika deer (Cervus nippon) of northern Japan. Jpn. J. Zoo Wildl. Med. 1:42–44.
8. Kato, H., K. Sato, Y. Ishikawa, S. Takahashi, Y. Gonai, and K. Yatsu. 1988. Papular stomatitis with dyscytologicalin Japanese serows, Capricornis crispus, in captivity. J. Jpn. Assoc. Zool. Gard. Aqua. 22:56–60. (In Japane.)
9. Kelly, P. J., M. Tagwira, L. Matthewman, P. R. Mason, and E. F. Wright. 1993. Reaction of sera from laboratory, domestic and wild animals in Africa with protein A and a recombinant chimeric protein AG. Comp. Immunol. Microbiol. Infect. Dis. 16:299–305.
9a. Komagai, T., et al. 1979. Abstracts of the 87th Meeting of the Japanese Society of Veterinary Science.
10. Kuroda, Y., M. Yoshida, T. Shibahara, T. Matsui, T. Nakane, H. Hara, Y. Insoshima, and H. Sentui. 1991. An epidemic of parapoxvirus infection among cattle: isolation and antibody survey. J. Vet. Med. Sci., in press.
11. 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. Vet. Immunol. Immunopathol. 28:247–258.
12. Mayr, A., and M. Böttner. 1990. Milker's node virus, p. 29–32. In Z. Dinter and B. Morein (ed.), Virus infections of ruminants. Elsevier Science Publishers, Amsterdam, The Netherlands.
13. Memar, O., and S. K. Tyring. 1995. Cutaneous viral infections. J. Am. Acad. Dermatol. 38:279–287.
14. Mercer, A., S. Fleming, A. Robinson, P. Nettleton, and H. Reid. 1997. Molecular genetic analyses of parapoxviruses pathogenic for humans. Arch. Virol. 13(1):113–128.
15. Moss, B. 1996. Parapoxviridae: the viruses and their replication, p. 2637–2671. In B. N. Fields, D. M. Knipe, and P. M. Howley (ed.), Fields virology, 3rd ed. Lippincott-Raven Publishers, Philadelphia, Pa.
16. Nagington, J., J. J. F. Obijeski, and J. H. Nakano. 1978. Current status of orthopoxvirus DNA: strain differentiation by electrophoresis of restriction endonuclease fragmented virion DNA. Virology 89:53–66.
17. Okada, H., M. Okada, S. Numakunai, and K. Ohshima. 1984. Electron microscopy on mucosal and cutaneous lesions in contagious papular dermatitis of Japanese serow (Capricornis crispus). Jpn. J. Vet. Sci. 46:297–302.
18. Okada, H. M., K. Okada, S. Numakunai, and K. Ohshima. 1984. Histopathologic studies on mucosal and cutaneous lesions in contagious papular dermatitis of Japanese serow (Capricornis crispus). Jpn. J. Vet. Sci. 46:257–264.
19. Robinson, A. J., and D. J. Lyttle. 1992. Parapoxvirus: their biology and potential as recombinant vaccines, p. 285–327. In M. Binns and G. L. Smith (ed.), Recombinant poxviruses. CRC Press, Inc., Boca Raton, Fla.
20. Robinson, A. J., and A. A. Mercer. 1995. Parapoxvirus of red deer: evidence for its inclusion as a new member in the genus parapoxvirus. Virology 208:812–815.
21. Rosenbusch, R. F., and D. E. Reed. 1983. Reaction of convalescent bovine anti sera with strain-specific antigens of parapoxviruses. Am. J. Vet. Res. 44:875–878.
22. Rossi, C. R., G. K. Kiesel, and M.-H. Jong. 1977. A paravaccinia virus isolated from cattle. Cornell Vet. 67:72–80.
23. Rubbel, G., H. Hönigsmann, and K. Wolff. 1982. The syndrome of milker's nodules in burn injury. J. Am. Acad. Dermatol. 63:334–339.
24. Sentui, H., T. Nishimori, I. Nagai, and N. Nishioka. 1996. Detection of sheep-associated malignant catarrhal fever virus antibodies by complement fixation tests. J. Vet. Med. Sci. 58:1–5.
25. Smith, K. J., H. G. Shelton III, W. D. James, and G. P. Lupton. 1991. Parapoxvirus infections acquired after exposure to wildlife. Arch. Dermatol. 127:79–82.
26. Suzuki, T., N. Minamoto, M. Sugiyama, T. Kinjo, Y. Suzuki, M. Sugimura, and Y. Atomi. 1993. Isolation and antibody prevalence of a parapoxvirus in wild Japanese serows (Capricornis crispus). J. Wildl. Dis. 29:384–389.
27. Suzuki, Y., M. Sugimura, Y. Atomi, N. Minamoto, and T. Kinjo. 1986. Wide spread of parapoxvirus infection in wild Japanese serows, Capricornis crispus. Jpn. J. Vet. Sci. 48:1279–1282.
28. Takatori, I., et al. 1980. Abstracts of the 98th Meeting of the Japanese Society of Veterinary Science.
29. Wittke, R., M. Herlyn, D. Schümer, P. A. Bachmann, A. Mayr, and R. Wyler. 1980. Genetic and antigenic heterogeneity of different parapoxvirus strains. Interwirology 13:33–41.
30. Yamagami, T., K. Takahashi, M. Sugiyama, K. Uematsu, Y. Noguchi, M. Haritani, and Y. Sudo. 1996. Parapoxvirus infection of wild Japanese serows (Capricornis crispus) in Tokyo. J. Jpn. Vet. Med. Assoc. 49:257–259. (In Japanese with English summary.)
31. Yamaguchi, T., K. Shirato, H. Fukushi, N. Minamoto, T. Kinjo, and K. Hirai. 1998. Prevalence of infectious agents, drug-resistant Escherichia coli and residual organochlorine in wild animals inhabiting the mountainous areas of central Japan. Jpn. J. Zoo Wildl. Med. 3:1–7. (In Japanese with English summary.)
32. Yata, S., T. Murakami, T. Ozawa, and H. Kitano. 1996. A case of parapoxvirus infection in wild Japanese serow (Capricornis crispus) in Ishikawa prefecture. Jpn. J. Zoo Wildl. Med. 19:93–97. (In Japanese with English summary.)