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SEROLOGICAL SURVEY OF THE IRIOMOTE CAT (FELIS IRIOMOTENSIS) IN JAPAN

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ABSTRACT: The Iriomote cat (*Felis iriomotensis*) was first discovered on Iriomote Island in the Yaeyama Islands of Japan in 1965. Ten male and 11 female adult cats were captured during the 6 yr period from 1983 to 1988. These were examined for evidence of viral and mycoplasmal infections. Neither *Mycoplasma* sp. nor *Ureaplasma* sp. were detected in swab samples of oropharyngeal and urogenital regions. A foamy virus was isolated from the oropharyngeal swab of a female cat examined in 1988. Feline leukemia virus was not detected in any of the cats. All cats were negative for serum antibodies to feline panleukopenia virus, feline herpesvirus, feline immunodeficiency virus and rotavirus. Eleven of 19 (58%), 14 of 17 (82%) and 6 of 17 cats (35%) had serum antibodies against feline calicivirus, coronavirus and feline syncytium forming virus, respectively.

Key words: Iriomote cat, Felis iriomotensis, wild cat, feline syncytium forming virus, foamy virus, calicivirus, coronavirus, feline leukemia virus, feline immunodeficiency virus, serological survey.

INTRODUCTION

The Iriomote cat (Felis iriomotensis) (Fig. 1) inhabits Iriomote Island in the Yaeyama Islands which lie at the southern end of the Nansei Islands of Japan. The island appears to be the only habitat for this species in the world. The cat was discovered in 1965 and it was formerly classified as Mayailurus iriomotensis (Imaizumi, 1967). Since then the cat has been designated as a special natural monument of Japan. There is no captive population of these animals on the island and they are all free-roaming in the jungle. The Environmental Agency of Japan (Kasumigaseki 1-2-2, Chiyoda-ku, Tokyo 100, Japan) has conducted ecological, morphological and behavioral studies on the Iriomote cat since 1974 and it has been estimated that only 80 to 100 of these animals exist on the island (Ono, 1985). The Iriomote cat is believed to be on the verge of extinction.

Akuzawa et al. (1987) described the results of hematological and parasitological study performed from 1983 to 1985 under

the Research Program for Preservation of Habitat of the Iriomote cat conducted by the Environmental Agency of Japan, the Laboratory of Ecology of Kyushu University (Hakozaki 6-10-1, Higashi-ku, Fukuoka 812, Japan) and the World Wildlife Fund Japan (Azabudai 2-4-5, Minato-ku, Tokyo 106, Japan). Hematological parameters of the Iriomote cat were similar to normal values in the domestic cat. Three digenean, two cestode, eight nematode and two coccidian species were found in the feces. Serum antibody against Toxoplasma gondii was detected in four of 16 cats examined. According to preliminary examinations of 11 cats captured during 1983-1984 (Mochizuki et al., 1985), the Iriomote cats were considered not to have been infected with most of the highly pathogenic microorganisms detected frequently in domestic cats. As opportunity has permitted, we have continued our examinations of Iriomote cats by using improved microbiological techniques. In the present report, we describe the results of viral and mycoplasmal examinations of 21 adult cats captured from 1983 to 1988.

MATERIALS AND METHODS

Sampling

Ten male and 11 female adult Iriomote cats were captured using a previously described box trap (Izawa et al., 1985; Akuzawa et al., 1987) from October 1983 to March 1988 by the permission of the Environmental Agency and the Agency for Cultural Affairs of Japan (Kasumigaseki 3-2-2, Chiyoda-ku, Tokyo 100, Japan). After the cats were premedicated by intramuscular (i.m.) administration of atropine sulphate (0.1 mg/cat; Atropine Sulphate Injection TA-NABE, Tanabe Pharmalogicals Ltd., Osaka, Japan), they were immobilized by i.m. administration of a combination of xylazine hydrochloride (1 mg/kg; Rompun, Bayer, Leverkusen, Federal Republic of Germany) and ketamine hydrochloride (5 mg/kg; Ketalar 50, Sankyo Ltd., Tokyo, Japan). After being anesthetized, the cats were weighed and inspected for general physical condition. Blood was taken from the jugular vein; serum was separated by low speedcentrifugation (3,000 rpm for 10 min) and stored at -20 C. Swabs for virology were collected from the oropharynx and nasal cavity; these were placed in a vial containing a transport medium which consisted of Eagle's minimum essential medium (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan) containing 5% fetal calf serum (GIBCO Lab., Grand Island, New York 14072, USA) and antibiotics (100 IU of penicillin G, 100 μ g of streptomycin and 5 μ g of amphotericin B/ml) and stored at -20 C. Swabs for mycoplasma examination were collected from oropharyngeal and urogenital sites (around the glans of penis or in vagina); these were placed in a vial containing a transport medium which consisted of Hayflick's medium supplemented with Agar Noble (0.15%; Difco Lab., Detroit, Michigan 48232, USA) and stored at 4 C. The samples for virology were transported to the laboratory of Kagoshima University (Kagoshima 890, Japan) and others for mycoplasmal examinations were sent to the laboratory of Miyazaki University (Miyazaki 889-21, Japan) either chilled in an icebox or frozen in dry ice. Following full recovery from anesthesia, animals were released at the site of their capture.

Virology and mycoplasmology

Virus isolation from the swab samples was performed using Crandell feline kidney cells (CRFK; Crandell et al., 1973). Samples from cats W-13 through W-17 were examined by using feline embryonic fibroblasts cells (FEA) which was a cell line developed in our laboratory as well. Cell culture medium and virus isolation methods were the same as previously described (Mochizuki et al., 1977; Mochizuki



FIGURE 1. A picture of the Iriomote cat (Felis iriomotensis) in the jungle.

and Konishi, 1979). Plaque reduction neutralization test (PRNT), micro-immunodiffusion (MID) test, rabbit anti-feline syncytium forming virus (FeSFV) immune serum, FeSFV Coleman strain and FeSFV S7801 strain isolated from the domestic cat in Japan (Mochizuki and Konishi, 1979) were used for virus identification of a virus isolated from the oropharynx of the Iriomote cat W-15 by the methods described previously (Gaskin and Gillespie, 1973; Mochizuki and Konishi, 1979).

Peripheral blood was examined for feline leukemia virus (FeLV) by the FeLV diagnostic (enzyme-linked immunosorbent assay: ELISA) test kit (Leukassay F[®] II; Pitman-Moore, Inc., Washington Crossing, New Jersey 08560, USA). When the test kit indicated FeLV-positive, the serum was retested by the focus inducing assay (FIA) improved by Jarrett et al. (1982) for detection of infectious FeLV. Furthermore, the serum was incubated with an equal volume of rabbit anti-FeLV p27 serum for 1 hr at room temperature and subsequently for overnight at 4 C, and then the mixture was re-examined by the Leukassay F* II (p27-neutralization assay: Lopez, 1988). The specificity of the antiserum was described previously (Mochizuki and Jarrett, 1985).

Detection of *Mycoplasma* sp. and *Ureaplasma* sp. from the swab samples was performed using Taylor-Robinson's medium for ureaplasma and Hayflick's medium for mycoplasma with slight modification (Nagatomo et al., 1982).

Serology

Serum antibody against feline panleukopenia virus (FPLV) was determined by hemagglutination-inhibition (HI) test with FPLV TU 1 strain described elsewhere (Konishi et al., 1975). In the HI test, formalin-fixed porcine erythrocytes were prepared by the method of Mathys et al. (1983), and the serum was pretreated by erythrocyte adsorption, kaolin and heat inactivation according to the method described by Wosu (1984). The HI antibody titer of more than 1:10 was regarded as positive.

Serum-neutralization (SN) antibodies against feline herpesvirus (FHV) C7301 strain (Mochizuki et al., 1977) and feline calicivirus (FCV) F2 strain (Takahashi et al., 1971) were examined by the microneutralization test described elsewhere (Mochizuki et al., 1987a) and antibody titers of more than 1:4 were regarded as positive.

Antibody against feline immunodeficiency virus (FIV) was examined by ELISA using the Petaluma strain of FIV infected CRFK cells (Pedersen et al., 1987; Yamamoto et al., 1988) as substrate antigens. The FIV infected CRFK cells were cultured on the coverslips (24×12) mm) for 24 hr and the coverslips were washed, fixed and stored by the method described by Yamamoto et al. (1988). Uninfected, normal CRFK cells were also prepared by the same procedure and were used as a negative antigen control. One hundred μ l of serum was mixed with 3.9 ml of phosphate-buffered saline solution (PBS), pH 7.4, in which 2×10^6 normal CRFK cells were suspended, and then were allowed to stand overnight at 4 C. After the low speed-centrifugation, the supernatant (1:40 dilution) was diluted serially twofold in PBS and were overlayered on the fixed cells. They were incubated at 37 C in a humidified chamber for 1 hr. The coverslips were washed for 15 min in 3 changes of PBS, and the cells were overlayered with a 1:800 dilution of goat biotinylated anticat IgG (H + L) immunoglobulin (Vector Lab. Inc., Burlingame, California 94010, USA). After 30 min incubation in the chamber at room temperature and the washing with PBS, the cells were stained by using STRAVIGEN B-SA Kit (BioGenex Lab., San Ramon, California 94583, USA). Anti-FIV positive and negative domestic cat sera supplied from Hiroyuki Koyama (Department of Veterinary Infectious Diseases, Kitasato University, Towada-shi, Aomori 034, Japan) always were included in the test as references. The serum samples were considered to be positive for FIV antibody activity when only the cytoplasm of infected CRFK cells, but not of uninfected CRFK cells was stained as in the case of positive reference serum. The titer of more than 1:40 was regarded as positive according to the report of Lutz et al. (1988).

Reverse passive HI (RPHI) antibody against a canine rotavirus RS 15 strain (Mochizuki and Hsuan, 1984) were examined and a titer of more than 1:10 was regarded as positive. The method of RPHI test was the same as that reported previously (Sanekata et al., 1982). Anti-coronavirus antibody was determined by ELISA using canine coronavirus (CCV)-infected CRFK cells as substrate antigen (Mochizuki and Furukawa, 1989). The ELISA titer of more than 1:10 was regarded as positive.

Micro-immunodiffusion test for serum antibody against FeSFV S7801 strain was performed according to Gaskin and Gillespie (1973).

RESULTS

Virology

Cytopathogenic viruses were not isolated from the swab samples of the cats tested except for cat W-15 as shown in Table 1. On the 3rd day after the first blind passage, slight cytopathic effect (CPE) was observed in the FEA cell culture to which the oropharyngeal swab sample of cat W-15 was inoculated. This CPE-producing agent (designated as ICFV W-15 strain) could be serially passaged in both FEA and CRFK cell cultures and it produced numerous multi-nucleated giant cells. The ICFV W-15 strain exhibited the same antigenicity as FeSFV strains in the MID test and MID antibody against FeSFV was presented in this cat's serum. Table 2 shows the serological relationship between Fe-SFV strains and the ICFV W-15 strain by PRNT. An antigenic difference was not detected between them by neutralization assay. The ICFV W-15 strain was consequently identified as foamy virus based on its biological and serological characteristics.

Only serum from cat W-15 was positive by the FeLV diagnostic ELISA kit (Table 1). However, no infectious FeLV was detected by the FIA, nor was the reaction inhibited by the rabbit immune serum specific for FeLV p27.

Mycoplasmology

Neither Mycoplasma spp. nor Ureaplasma spp. was isolated from the swab samples collected from the Iriomote cats.

Anti-virus antibodies

On the cats captured during 1983–1984, the HI antibody titer against FPLV, the

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ate of posi	itive tit	Rate of positive titer of viral antibody				%0	%0	58% (21) ^h	%0	82% (108)	35%			

"O, oropharyngeal swab; N, nasal swab; U, urogenital swab.

FeLV, feline leukemia virus; FPLV, feline panleukopenia virus; FHV, feline herpesvirus; FCV, feline calicivirus; CCV, canine coronavirus; FeSFV, feline syncytium forming virus; FIV, feline immunodeficiency virus.
⁴ ELISA, enzyme-linked immunosorbent assay; HI, hemagglutination-inhibition; SN, serum neutralization; RPHI, reverse passive HI; MID, micro-immunodiffusion.

- , negative: +, positive.
NT, not tested: NA, not available; NSI, non-specific inhibition.
The cat designated W-5 was captured and examined totally three times during 1983 to 1985.
Numbers in parentheses are geometric mean titer of antibody-positive cats.

TABLE 2. Serological relationship between feline syncytium forming virus (FeSFV) strains and the foamy virus (ICFV W-15) strain isolated from the Iriomote cat by plaque reduction neutralization test.

Virus	Anti- FeSFV immune serum ⁴	W-5-3 serum	W-15 serum
ICFV W-15 strain	160 ⁵	80	80
FeSFV Coleman strain	320	40	40
FeSFV S7801 strain	320	80	40

* Anti-FeSFV Coleman strain immune serum was prepared by using rabbits (Mochizuki and Konishi, 1979).

^b Plaque reduction neutralization titer was expressed as the reciprocal of the highest serum dilution that reduced the number of plaques by 50% or more.

SN antibody titers against FHV and FCV, and the RPHI titer against the canine rotavirus had been already presented in our preliminary report (Mochizuki et al., 1985), which is cited in Table 1. The serum of cat W-6 could not be evaluated at a titer of 1:10 in the HI test of FPLV because of the lack of an adequate serum sample. No antibody against FPLV, FHV, FIV or rotavirus were found in the sera of the cats we examined. However, 11 of 19 (58%) cats had SN antibody against FCV at titers which ranged from 1:2 to 1:256 with the geometric mean (GM) antibody titer of 1:21. Fourteen of 17 (82%) cats had ELISA antibody against CCV at titers which ranged from 1:10 to 1:1,280 with the GM antibody titer of 1:108. Antibody against FeSFV was detected in 6 of 17 (35%) cats examined as shown in Table 1.

DISCUSSION

The foamy virus cultured in the present study is the first virus isolated from the Iriomote cat. This ICFV W-15 strain was antigenically not distinct from FeSFV of domestic cats. It infected domestic cats with no clinical manifestation and normal hemograms (M. Mochizuki and I. Kawaji, unpubl. data). The foamy virus, which is classified into the subfamily Spumavirinae of the Retroviridae, has been reported in a number of mammalian species (human, monkey, cattle, cat and hamster) and may induce persistent infections without evident pathogenesis in their natural host (Hooks and Gibbs, 1975; Teich, 1984). The foamy viruses have been isolated from other wild cats such as the European wildcat (Felis sylvestris; Lieber et al., 1975) and leopard cat (Felis bengalensis; Rasheed and Gardner, 1981). Anti-FeSFV antibody was detected in about 35% of the Iriomote cats examined (3 of 10 males tested; 3 of 7 females tested). The antibody-positive rate in this study was higher than the rate (4%)determined in a serological survey of domestic cats in the Tokyo area a decade ago (Mochizuki and Konishi, 1979) and the result of our recent examination of the domestic cats in Japan (n = 178, average of 14%, 24% males, 12% females, M. Mochizuki and I. Kawaji, unpubl. data). The numbers of antibody-positive Iriomote cats (n = 6) is small and developing any hypotheses of this virus infection is tenuous. However, the higher prevalence (43%) of female Iriomote cats compared with that reported (12%) in female domestic cats may suggest that the foamy virus infection has been spread in the Iriomote cat population not only by bites but also by transmission through milk and maternal grooming from the infected females. Feline syncytium forming virus is not thought to be responsible for any clinical illness in domestic cats but has been statistically linked to chronic progressive polyarthritis in which FeLV may function in its pathogenesis, or it may potentiate FeSFV infection by altering the host's immune response (Pedersen et al., 1980). Therefore, the foamy virus infection is not considered to be an important cause of illness of the Iriomote cat, especially since FeLV infection has not been documented.

Evidence of FeLV infection in captive nondomestic cats has been documented (Rasheed and Gardner, 1981; Meric, 1984; Briggs and Ott, 1986; Citino, 1986) and a type C virus closely related to feline endogenous virus RD-114 also was isolated from a European wildcat (Lieber et al., 1975). However, we know of no previous reports of FeLV isolation from true wild felids like the Iriomote cat. Infectious FeLV was not detected by the FIA or by specific inhibition by the p27-neutralization assay; therefore, the positive response exhibited in the serum of one cat by the FeLV diagnostic ELISA kit must be a false-positive reaction. In the study of Florida panthers (Felis concolor corvi), Lopez (1988) successfully demonstrated that false-positive results obtained with some commercial FeLV detection kits were due to an elevation in antibody specific for mouse immunoglobulin which had been probably produced by administration of a mouse brain-origin, killed virus rabies vaccine. The cause of false-positive reactivity of cat W-15 serum remains uncertain.

Feline immunodeficiency virus, originally called feline T-lymphotropic lentivirus, also belongs to subfamily of Retroviridae, the Lentivirinae (Pedersen et al., 1987), and it is established that FIV is a major cause of a chronic acquired immunodeficiency-like syndrome in domestic cats (Pedersen et al., 1987; Yamamoto et al., 1988). Recently, Barr and Barlough (1989) described that evidence of FIV infection has been found in several species of nondomestic cats within zoo populations as well as in free-roaming populations of Florida panthers and bobcats (Felis rufus) found in and around the Everglades. In this survey, anti-FIV antibody was not demonstrated in sera of 17 Iriomote cats, and which indicates that FIV infection is not enzootic in the Iriomote cat population.

Two coronaviruses, feline infectious peritonitis virus (FIPV) and feline enteric coronavirus (FECV) infect domestic cats; FIPV causes a chronic, progressive, immunologically-mediated, fatal disease in both domestic and exotic Felidae (Pedersen, 1983; Evermann et al., 1988). They are closely related antigenically to CCV and transmissible gastroenteritis virus (TGEV) of swine (Pedersen et al., 1978, 1984). Domestic cats can be infected experimentally with CCV and TGEV which

elicit an antibody response with no clinical manifestation (Reynolds and Garwes, 1979; Barlough et al., 1984). Presently available serological tests by using homologous or heterologous viruses as substrate antigens do not differentiate between antibodies against FIPV, FECV, CCV or TGEV in cats (Barlough et al., 1983). In our recent testing of domestic cats by the ELISA assay applied here, all cats clinically suspected as FIP (n = 28) possessed antibody titer ranging from 1:10 to 1:3,276,800 with a GM titer of 1:3,735, however, antibody was detected in 81% of sera from clinically normal cats (n = 16) as well and the titers ranged from 1:10 to 1:1,280 with GM titer of 1:89 (M. Mochizuki, unpubl. data). The anti-coronavirus antibody titer is only used as an aid to the diagnosis of FIP in veterinary clinics. It was certain that coronavirus infection(s) were present in the Iriomote cat population based on the presence of ELISA antibody titers greater than 1:320 in some cats. There may be no serious infections in the Iriomote cats, however, because all animals we have examined were considered to be healthy based on the results of a general physical inspection and the hematological examination (Akuzawa et al., 1987; M. Akuzawa and M. Mochizuki, unpubl. data from five cats captured in 1988). The positive serologies may reflect exposure to cross-reacting but less virulent coronaviruses of other species (possibly canine and swine) that the Iriomote cat encounters in nature. However, an epizootic of FIP in a captive cheetah (Acinoyx jubatus) population during 1982-1983 (Pfeifer et al., 1983) served to focus attention on the susceptibility of this endangered animal to infectious diseases with emphasis on FIP and the data obtained thus far provided the basis for a hypothesis that the cheetah, through intensive inbreeding, had become more susceptible to viral infections as a result of genetic homogeneity and there might be the cheetah coronavirus distinct from FIPV and FECV in nature (Evermann et al., 1988). Furthermore, a high percentage of

wild-caught cheetahs in southern and eastern Africa possessed anti-coronavirus antibodies (Horzinek and Osterhaus, 1979; Evermann et al., 1988). Obviously, there is a need to continue serological surveillance of the Iriomote cat.

Both FCV and FHV are primary pathogens in the respiratory tract of domestic cats, and they have been occasionally associated with illness in other felids (Sabine and Hyne, 1970; Boever et al., 1977). In general, a carrier state with virus excretion from the oropharynx is believed to be the normal sequel to FCV infection in domestic cats (Wardley and Povey, 1976). However, calicivirus or other antigenically related viruses were not recovered from the oropharyngeal swabs in spite of the evidence that about one half of the cats possessed anti-FCV neutralizing activity. Obviously additional detailed studies are needed to clear the significance of this anti-FCV activity detected.

Feline panleukopenia is an acute parvovirus disease of probably all members of Felidae and some other hosts; it is characterized by severe enteritis and lymphopenia with high mortality especially in nonimmune kittens (for a review see Gillespie and Scott, 1973; Povey, 1985). We are concerned about the result that anti-FPLV antibody was not found in any cats. Although it is unknown whether or not the Iriomote cat is susceptible to FPLV, if they are as we suspect, there have been no FPLV epizootics in the population and the cats have no immunological resistance to FPLV infection. While some common cat viruses such as FeLV, FIPV, FIV and FHV are labile in the environment, FPLV is shed in feces, urine and saliva of acutely infected domestic cats; it can survive in the environment as a source of infection for other animal species for months.

On the island where the Iriomote cats live, there are also feral swine (Sus scofa), rabbits (Sylvilagus spp.), several species of rodents and a small number of feral deer (Cervus spp.), cattle and goats, but there may be no other wild felids. Cattle, water

buffaloes, goats, swine, and horses are raised in addition to domestic cats and dogs. The ecological relationship between the Iriomote cats and free-roaming domestic cats and dogs has not been investigated. The Iriomote cats usually avoid human habitations and it is presumed that they seldom come into contact with domestic cats. However, there are a few incidents in which the Iriomote cats raided henhouses for chickens or captured goat kids in the pasture. Therefore, it is possible that indirect transmission of some domestic animals' pathogens to the Iriomote cat may occur. Evidence of FeLV and FIV infections has not been documented in the Iriomote cats, but a foamy virus indistinguishable from FeSFV has been isolated and anti-FeSFV antibodies have been found in 35% of the cats in the present survey. These retroviruses are secreted in saliva of persistently infected cats and fighting (mainly by bites) is believed to be one of the primary routes of their transmission (Francis et al., 1977; Pedersen, 1987; Yamamoto et al., 1989). There has been a strong correlation between FeSFV and FIV infections in domestic cats, probably because of the common mode of transmission (Yamamoto et al., 1989). Although their prevalence in the domestic cats on the island has not been studied yet, the results of the present survey suggest that the Iriomote cat is largely isolated spatially from the domestic cat and that the foamy virus has been retained in its population rather than recently introduced from domestic cats. In conclusion, we consider that the Iriomote cats have not been infected vet with devastating domestic feline pathogens. We believe that the best management for preventing these diseases in this endangered species is not to import any diseased and/ or additional superfluous domestic cats onto the island.

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