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Molecular epidemiology of *Microsporum canis* isolated from Japanese cats, dogs and pet owners by multilocus microsatellite typing fragment analysis.

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Summary

Multilocus microsatellite typing (MLMT) was performed on 93 strains of *Microsporum canis* isolated between 2012 and 2017 from 75 cats, eight dogs and 10 pet owners. These strains were derived from two major reservoirs: commercial breeding facilities and pet shops (PS), and stray cats and pet cats that went outdoors and came in contact with stray cats (Outdoor). Six microsatellite markers were used for genotyping. These 93 strains included 22 genotypes, 11 previously detected in Japan and 11 new genotypes. Strains belonging to the previously reported genotypes P and A were distributed widely throughout Japan. Genotype P was the most frequent, accounting for 37 (39.8%) of the 93 strains, most derived from Outdoor sources. Genotype A was the second most frequent, being present in 11 (11.8%) of the 93 strains, most derived from the PS reservoir. All new genotypes were detected in isolates from cats, with many of these derived from the Outdoor reservoir. The consistency of infection was shown in 18 of 19 familial cases. These findings indicate that genotypes differ in strains derived from PS and Outdoor reservoirs and that genotyping by MLMT is useful for tracking the routes of spread and transmission of *M. canis* in Japan.

Introduction

The zoophilic dermatophyte *Microsporum canis* (*M. canis*) is one of the most common human pathogens causing tinea capitis and tinea corporis (1,2). *M. canis* invaded Honshu, the main island of Japan, in the 1950s through the importation of contaminated purebred pet cats (3). In the 1970s and 1980s, Japan experienced a nationwide epidemic of human dermatophytosis caused by *M. canis* (1). Initially, the fungus was thought to spread among purebred cats at pet shops and breeding facilities. Contaminated cats sold to customers that went outside their homes spread the fungus to similar pet cats and to communities of stray cats (3-5), which became the main sources of human infection (3,6-8).

Our recent epidemiological studies of dermatophytosis of pet animals in Japan (9,10) showed that *M. canis* was the most prevalent causative fungus in both cats and dogs. In cats, there are two major reservoirs of the fungus: pet shops and commercial breeding facilities (PS), and stray cats and pet cats that go outdoors and come in contact with stray cats (Outdoor). In dogs, there is only one reservoir, the PS route (10). However, the infection status of the fungus among the reservoirs and risk of contagion to humans are still unclear.

Recent advances in molecular biology have enabled intraspecies genotyping (11), and molecular markers for *M. canis* have been evaluated to trace the routes of infection and analyze fungal pathogenicity (12-15). Molecular markers that have been assessed include polymorphisms of

microsatellite (MS) DNAs (14,15), which consist of several short, tandemly repeating DNA sequences, with strain discrimination determined by analyzing differences in the number of repeats, a process called multilocus microsatellite typing (MLMT). An MLMT investigation of the molecular polymorphisms in 59 *M. canis* strains isolated from human dermatophytoses and 11 strains isolated from cats in Japan found that these 70 strains could be divided into 20 genotypes, named genotypes A to T (16).

The present study was designed to determine the MLMT genotypes of *M. canis* strains isolated in recent years from feline and canine dermatophytoses in Japan and to evaluate the roles of these animals as reservoirs of *M. canis* (10).

Materials and Methods

Fungal strains

The present study evaluated 93 strains of *M. canis* isolated in Japan from September 2012 to April 2017 (Table 1). Of these 93 strains, 72 were isolated from 72 cats with clinical symptoms of dermatophytosis, three were isolated from three healthy looking cats cohabiting with cats with dermatophytosis, eight were isolated from eight dogs with dermatophytosis, and ten were isolated from the owners of infected pet animals included in this study. Fungal isolates from infected cats, dogs, and their owners were grown on Mycosel® agar plates (Eiken Kagaku Co., Tokyo), with one typical colony subjected to MLMT. Healthy looking cats were sampled by rubbing the entire body of each with a

cotton swab (9), followed by culture of their tips on Mycosel[®] agar plates. Species identification was based on morphological characteristics and restriction fragment length polymorphisms (RFLP) of internal transcribed spacer (ITS) regions of ribosomal DNA following digestion with the restriction enzymes *MvaI* (17) and *HinfI* (18).

To determine whether an animal had been infected by one genotype or multiple genotypes at the same time, isolates from three infected cats were cultured on agar plates, and 10 colonies were randomly picked from each plate. The fungal strains were collected by veterinarians from five regions in Japan, i.e., Kanto, Hokuriku, Kinki, and Chugoku on Honshu, the largest island in Japan, and Shikoku island. Despite requests, however, fungal strains were not collected from other parts of Japan. Sampling from animals and owners, and documenting of the origin of the animals was approved by the Ethics Committee of Kanazawa Medical University (Approval No. I543).

Based on information provided by pet owners and an epidemiological study of feline and canine dermatophytosis in Japan (10), fungal origins were divided into two routes, i.e., PS and Outdoor.

Preparation of template DNA

Fungal DNA was extracted from colonies grown on Sabouraud dextrose agar slants or plates, as described (16), with slight modifications. Briefly, small amounts of mycelial mat rinsed with 70% ethanol were harvested and ground in 200 µl lysis buffer (200 mM Tris-HCl, pH 7.5, 0.5% SDS, 250 mM NaCl, 25 mM EDTA). The homogenates were heated at 100°C for 5 min, followed by the addition of 100 µl 3 M sodium acetate, pH 7.0. The homogenates were placed on ice for 5 min and centrifuged,

and 300 µl of isopropanol were added to each supernatant. After centrifugation, the DNA pellets were washed in 70% ethanol, dried, and dissolved in 100 µl of 10 mM Tris-HCl (pH 8.0) solution. The template DNAs were used for the RFLP analysis of ITS regions (17,18) and for MLMT analysis.

MLMT analysis

1) Primers used in MLMT analysis

Six previously described MS markers (Table 2) (16) were used in this study. Forward primers were labeled with one of the following fluorescent dyes: 6-FAMTM, VIC®, PET®, or NEDTM (Applied Biosystems, Tokyo, Japan). To avoid plus A and stutter peaks, which can contaminate non-template peaks, thereby complicating the identification of true peaks, a tail was added to the 5' end of each reverse primer (16). All customized primer pairs were synthesized and supplied by Life Technologies Japan Ltd. (Tokyo, Japan).

2) PCR and MLMT

PCR and MLMT were performed as described (16), with slight modifications. PCR amplification of genomic DNA was performed in a reaction mixture (20 µL) containing 1 µL template DNA, 10 µL GoTaq® Green Master Mix (Promega, Madison, WI, USA), and 20 pmol of each primer. The amplification protocol consisted of an initial denaturation at 95°C for 5 min; followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 1 min; followed by a final extension at 72°C for 6 min. To simplify the method, the annealing temperatures in the original studies (54, 56, and 58°C) for all six PCR amplifications (Table 2) (15,16) were fixed at

55°C. Preliminary experiments using *M. canis* strain CBS113480 confirmed that the yield of each PCR product when the annealing temperature was set at 55°C was equivalent to that obtained when PCR amplifications utilized the original annealing temperatures (15) (data not shown). PCR products were diluted 1:20 or 1:40 with distilled water, and a 1 µL aliquot of each mixture was added to 10 µL Hi-Di™ formamide (Applied Biosystems), along with a 0.2 µL aliquot of size markers (GeneScan™ 600 LIZ® size standard, Applied Biosystems). The samples were denatured for 5 min at 95°C and applied to an ABI PRISM™ 3500XL Genetic Analyzer (Applied Biosystems). The main peaks were determined using GeneMapper® ver. 4.0 software (Applied Biosystems). The new genotypes detected in this study were labeled K1 through K11.

Results

Genotyping of isolates from individual animals.

All 10 strains selected from each of three infected cats, Nos. 61, 62 and 63, were of the same genotype; i.e., genotypes K8, P, and L, respectively (Table 1). One representative strain from each of these cats, KMU 9712 from No. 61, KMU 9722 from No. 62 and KMU 9732 from No. 63, was included in the epidemiologic analyses.

Genotype differentiation by MLMT

All the strains revealed the same peak when PCR was performed with primers MS5, whereas variations were found when PCR was performed with the other primers, including two molecular

variations each with MS1 and MS6, four each with MS2 and MS 7, and 12 with MS4. Of the 93 strains, 91 showed single peaks for each of six markers. However, KMU 8831 isolated from No. 3 and KMU 8835 from No. 7 each generated two peaks with MS6, at 115 bp and 129 bp (Table 1, Figure 1). To clarify the origin of these peaks, the sizes of amplicons of eight subcultures derived from a homogenate of a colony of KMU 8831 were determined using the MS6 primer pair. Of these eight subcultures, seven showed a band corresponding to the peak at 115 bp, and one showed a band corresponding to the peak at 129 bp (Table 1, Figure 1), indicating that KMU 8831 had a complex genotype. The two peaks of KMU 8835 may have originated in the same manner. In combination with these variations, the 93 strains consisted of 22 genotypes, with 67 strains possessing 11 previously reported genotypes (genotypes A, B, D, F, G, H, I, L, P, Q, and S) (16) and 28 strains possessing 11 new genotypes (genotypes K1 to K11). Strains KMU 8831 and KMU 8835 were regarded as having two genotypes each (Table 1, Table 3). Genotype P was the most frequent, being present in 37 (39.8%) of the 93 strains, followed by genotypes A (11 genotypes, 11.8%), L, and K3 (5 genotypes each, 5.4%).

Genotyping of strains from familial outbreaks

Of the 93 strains, 47 were derived from 19 familial outbreaks (Table 3), 18 from cats and one from a dog. Of these 19 familial outbreaks, 18 showed a single genotype. In a familial outbreak case No.12, the animal regarded the initiator of infection (No.7) had two genotypes, KMU 8835 (genotype P) and a new genotype (K1), whereas a co-habiting animal (No. 6) had one of these genotypes (P; KMU 8834). Genotype P was again the most frequent, being present in seven (36.8%) of the 19

outbreaks, followed by genotypes A and K4 (2 outbreaks each, 10.5%).

Backgrounds of strains and genotyping.

Investigation of the relationships between the demographic characteristics of strains and genotypes (Table 4) showed that 38 (40.9%) of the 93 strains were of Outdoor origin, with two genotypes detected in each of the two strains. These 38 strains included 15 identified genotypes, five previously reported genotypes (P, A, L, H, and Q) and 10 newly detected genotypes (K1–7 and K9–11). Twenty-four (25.8%) of the 93 strains were derived from the PS reservoir and included 10 genotypes, including eight previously reported (genotypes A, P, S, F, G, I, L, B) and two new (genotype K8, K9) genotypes. Genotype P and A were found nationwide throughout Japan. Genotype P was the most frequent, accounting for 37 (39.8%) of the 93 strains, most derived from Outdoor sources, especially lived in Kanto and Hokuriku. Genotype A was the second most frequent, being present in 11 (11.8%) of the 93 strains, most derived from the PS reservoir in Hokuriku and Shikoku.

Discussion

Microsporium canis is a fungus with low intraspecific variation, with a very limited number of molecular markers available for intraspecies molecular typing and strain identification (12-15). Currently, MLMT is the most sensitive method of molecular typing and has been used to identify virulent strains (14) and to trace infections (15). This method was also shown to be useful to assess the molecular epidemiology of *M. canis* isolates, which were mainly from humans in Japan (16). To

investigate the current status of infection in Japan, this study subjected strains derived from dogs and pet owners, as well as from cats, to molecular genotyping.

In testing the discrimination potential of six MS markers, we found that one, MS5, was unable to identify any molecular polymorphisms, in agreement with a previous study from Japan (16). In contrast, a report describing 26 genotypes among isolates from 13 countries found three variations in MS5 products (15), indicating that MS5 markers should not be omitted. To determine the consistency of the MLMT genotype in each animal, 10 colonies were randomly selected from culture plates of three infected cats. None of these sets of colonies showed any variations in genotype, suggesting that selection of a typical colony from each plate for genotyping was not inappropriate. However, two strains, KMU8831 and 8835, derived from Outdoor animals that lived in the same environment, showed complex genotypes with P and K1. Coexisting genotypes on the same animal may occur if samples are selected separately from a colony cultured on one plate.

The MLMT method has been shown useful for tracing *M. canis* infection (15), and the consistency of genotypes infecting pet animals and their owners is of epidemiologic interest. Previous studies have shown genotype consistency in outbreaks (16, 19), and the present study supports these findings, because 18 of the 19 familial cases were caused by fungi with the same genotype. The other case consisted of two strains (KMU8831, 8835) isolated from two cats (No.3, 7) inhabiting the same temple and one strain (KMU8834) isolated from a cat (No.6) living with a cat

(No.7). Although two strains showed molecular variations, all three cats were infected with a strain of genotype P, showing consistency.

The relationship between MLMT genotypes and the virulence of *M. canis* suggested that certain phenotypes are virulent in humans (14), whereas another study using the same MS markers reported that there were no genotype differences in feline pathogenicity (20). In the present study, the frequencies of the genotypes that caused familial infections reflected the overall frequencies of these strains, with no genotype found associated with susceptibility to familial infection.

Genotype variation and its relationship to the origin of the strains are also of epidemiologic interest. Most of the strains assessed in this study were isolated from pet animals, with genotype frequency differing markedly from that of strains isolated mainly from humans (16). Genotype A was the most frequently observed human isolate (21.4%), followed by genotypes H (14.3%) and P (11.4%) (16). In the present study, genotype P (39.8%) was the most frequent, followed by genotype A (11.8%), with genotype H being present in only one of the 93 strains (1.1%). Differences in genotype distribution may also reflect geographical differences. Most of the strains isolated in the previous study (16) were from central to western Japan, whereas many of the strains in the present study were isolated from eastern Japan.

This study also confirmed that both genotypes P and A were widely distributed, although their origins differed. Most genotype P strains were isolated from Outdoor animals, whereas most genotype A strains were isolated from PS related animals. *Microsporium canis* invaded Japan in

1950s (3) and may have spread throughout the country by the movement of contaminated purebred cats, followed by its spread among stray cats and current maintenance in many communities of stray cats. Most genotype A strains, including three isolated from dogs, were of PS origin. Most cats and dogs sold at pet shops in Japan are bred at large animal breeding facilities, with genotype A being prevalent at these facilities and at pet shops. In addition, a high percentage of PS related strains are of genotypes other than P and L and may therefore be maintained in some breeding facilities. In contrast, the detection of genotype P in large numbers in outdoor animals was likely due to its detection in large numbers of animals in the Kanto and Hokuriku regions. Regional outbreaks may have occurred in these two regions at the time of the survey, or large communities of infected cats may be present in these regions. Ten (genotypes K1–K7, K9–K11) of the 11 new genotypes were detected among Outdoor strains. Each genotype was composed of a small number of strains. MS4 products were found to vary considerably, with 10 molecular variations observed among the 11 genotypes. These variations may be due to MS mutations that occurred after *M. canis* strains spread to outdoor animals. Microsatellite DNA sequences are known to be highly variable and to mutate at rates several orders of magnitude faster than SNP mutations (21). The high rate of the former can result in spontaneous mutations that form new length variants, which can be detected during pedigree analysis (22).

This study had limitations in the standardization of MLMT and sampling. Although standardization of facilities yielded the same results in two laboratories in Germany and Italy (15),

sharing MLMT data across laboratories may have limitations (21). Peaks may be affected by using different capillary gel electrophoresis equipment and PCR conditions (16), with strict calibration required for data sharing. For example, we expected that the MS5 product of CBS113480 would be 105 bp in length. However, we found that this product was 106 bp in length (16), and another study found that it was 100 bp in length (15). Sampling is also important. The facilities participating in this study did not cover all regions of Japan, and isolates collected by certain veterinarians interested in fungal infections were from limited areas in each region. Therefore sampling was biased and this survey may not accurately reflect the actual state of infection throughout the country. In addition, the present study indicated the presence of strains (cases) of complex genotypes, and examining multiple isolates from a primary culture may make more informative genotyping possible.

In conclusion, this study found that genotypes based on MLMT in Japanese strains of *M. canis* are more diverse than in a previous study (16), with reservoirs divided into PS and Outdoor. Genotyping is useful for better understanding route of transmission and the regional spread of infection.

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Conflicts of interest

None to declare.

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Legends

Fig. 1: Analysis of the strains with two peaks using MS6 primers.

A: Profiles by capillary electrophoresis of the amplicons of KMU 8831 and 8835. Two peaks each, at 115 bp and 129 bp, were observed in both KMU 8831 and KMU 8835.

B: Banding profiles of amplicons by acrylamide gel electrophoresis of KMU 8831 and eight randomly isolated subcultures derived from a homogenate of a colony of KMU 8831, along with amplicons of KMU 8834 and KMU 8835.

Table 1. Background and genotypes of the 93 individual *Microsporium canis* strains analyzed in this study.

Genotype	No. of each genotype	KMU No.*	Region	Year isolated	Source	Case No.	Route of infection‡	Background	Amplicon size (bp)					
									MS1	MS2	MS4	MS5	MS6	MS7
A	11	8832	Hokuriku	2012	Dog	4	PS	Purchased at a pet shop	118	106	108	106	115	131
A		8833	Hokuriku	2012	Dog	5	Co-habiting	Co-occurring infection from a dog (No. 4)	118	106	108	106	115	131
A		9078	Hokuriku	2013	Cat	12	PS	Purchased at a pet shop	118	106	108	106	115	131
A		9082	Hokuriku	2013	Cat	14	PS	Purchased at a pet shop	118	106	108	106	115	131
A		9605	Hokuriku	2015	Dog	38	PS	Purchased at a pet shop	118	106	108	106	115	131
A		9371	Kanto	2014	Cat	25	PS	Purchased at a pet shop	118	106	108	106	115	131
A		9744	Kanto	2015	Cat	65	Outdoor	Stray cat	118	106	108	106	115	131
A		9456	Shikoku	2015	Cat	28	PS	Purchased at a pet shop	118	106	108	106	115	131
A		9457	Shikoku	2015	Cat	29	PS	Purchased at a pet shop	118	106	108	106	115	131
A		9842	Shikoku	2016	Cat	74	PS	Purchased at a pet shop	118	106	108	106	115	131
A		10047	Shikoku	2016	Cat	86	PS	Purchased at a pet shop	118	106	108	106	115	131
B	1	10045	Chugoku	2016	Cat	84	PS	Purchased at a pet shop	118	108	108	106	115	131
D	1	9743	Hokuriku	2015	Cat	64	Others	Indoor infection†	118	106	125	106	115	127
F	2	9170	Kanto	2014	Cat	15	PS	Purchased at a pet shop	118	106	159	106	115	129
F		9473	Kanto	2015	Cat	30	PS	Purchased at a pet shop	118	106	159	106	115	129
G	1	9774	Kanto	2016	Cat	71	PS	Purchased at a pet shop	118	98	159	106	115	129
H	1	9012	Shikoku	2013	Cat	11	Outdoor	Stray cat	118	106	161	106	115	129
I	3	9770	Hokuriku	2015	Cat	69	PS	Purchased at a pet shop	118	106	165	106	115	129
I		9771	Hokuriku	2015	Cat	70	Co-habiting	Co-occurring infection from a cat (No. 69)	118	106	165	106	115	129
I		10132	Hokuriku	2017	Cat	93	PS	Purchased at a pet shop	118	106	165	106	115	129
L	5	9769	Hokuriku	2015	Cat	68	Outdoor	Stray cat	118	108	161	106	115	129
L		10044	Hokuriku	2016	Dog	83	PS	After shampooing at a pet shop	118	108	161	106	115	129
L		9217	Shikoku	2014	Cat	20	Outdoor	Household cat going out	118	108	161	106	115	129
L		9601	Shikoku	2015	Cat	35	Outdoor	Stray cat	118	108	161	106	115	129
L		9732	Shikoku	2015	Cat	63	Outdoor	Household cat going out	118	108	161	106	115	129
P	37	8990	Chugoku	2013	Cat	10	Outdoor	Stray cat	120	106	144	106	115	129
P		9602	Chugoku	2015	Cat	36	Outdoor	Stray cat	120	106	144	106	115	129
P		10041	Chugoku	2016	Dog	80	PS	Purchased at a pet shop	120	106	144	106	115	129
P		8750	Hokuriku	2012	Cat	1	PS	After shampooing at a pet shop	120	106	144	106	115	129
P		8751	Hokuriku	2012	Human	2	Co-habiting	Owner of a pet cat (No. 1)	120	106	144	106	115	129
P		8831†	Hokuriku	2012	Cat	3	Outdoor	Stray cat from the same temple as a cat(No.6)	120	106	144	106	115	129
P		8834	Hokuriku	2012	Cat	6	Outdoor	Stray cat from the same temple as a cat(No.3)	120	106	144	106	115	129
P		8835†	Hokuriku	2012	Cat	7	Co-habiting	Co-occurring infection from a cat (No. 6)	120	106	144	106	115	129
P		9079	Hokuriku	2013	Cat	13	Outdoor	Stray cat	120	106	144	106	115	129
P		9379	Hokuriku	2014	Cat	27	Outdoor	Stray cat	120	106	144	106	115	129
P		9632	Hokuriku	2015	Cat	42	Outdoor	Household cat treated with steroids, going out	120	106	144	106	115	129
P		9633	Hokuriku	2015	Human	43	Co-habiting	Owner of pet cats (Nos. 42, 44, 46)	120	106	144	106	115	129
P		9634	Hokuriku	2015	Cat	44	Co-habiting	Co-occurring infection from a cat (No. 42)	120	106	144	106	115	129
P		9637	Hokuriku	2015	Cat	46	Co-habiting	Co-occurring infection from a cat (No. 42)	120	106	144	106	115	129
P		9171	Kanto	2014	Cat	16	Outdoor	Household cat going out	120	106	144	106	115	129
P		9181	Kanto	2014	Human	17	Co-habiting	Owner of a pet cat (No. 16)	120	106	144	106	115	129
P		9289	Kanto	2014	Cat	21	Outdoor	Household cat going out	120	106	144	106	115	129
P		9321	Kanto	2014	Cat	22	Co-habiting	Co-occurring infection from a unknown stray cat	120	106	144	106	115	129
P		9378	Kanto	2014	Cat	26	Outdoor	Stray cat	120	106	144	106	115	129
P		9495	Kanto	2014	Cat	32	Outdoor	Stray cat	120	106	144	106	115	129
P		9496	Kanto	2015	Human	33	Co-habiting	Owner of the pet cat (No.32)	120	106	144	106	115	129
P		9603	Kanto	2015	Cat	37	Co-habiting	Co-occurring infection from a cat (No. 32)	120	106	144	106	115	129
P		9658	Kanto	2015	Cat	47	Outdoor	Household cat going out	120	106	144	106	115	129
P		9662	Kanto	2015	Cat	50	Outdoor	Stray cat	120	106	144	106	115	129
P		9664	Kanto	2015	Cat	52	Outdoor	Stray cat	120	106	144	106	115	129
P		9675	Kanto	2015	Cat	53	Outdoor	Stray cat	120	106	144	106	115	129
P		9676	Kanto	2015	Cat	54	Co-habiting	Co-occurring infection from a cat (No. 53)	120	106	144	106	115	129
P		9677	Kanto	2015	Cat	55	Co-habiting	Co-occurring infection from a cat (No. 53)	120	106	144	106	115	129
P		9678	Kanto	2015	Cat	56	Co-habiting	Co-occurring infection from a cat (No. 53)	120	106	144	106	115	129
P		9679	Kanto	2015	Cat	57	Co-habiting	Co-occurring infection from a cat (No. 53)	120	106	144	106	115	129
P		9680	Kanto	2015	Cat	58	Co-habiting	Co-occurring infection from a cat (No. 53)	120	106	144	106	115	129
P		9681	Kanto	2015	Cat	59	Co-habiting	Co-occurring infection from a cat (No. 53)	120	106	144	106	115	129
P		9722	Kanto	2015	Cat	62	PS	Purchased at a pet shop	120	106	144	106	115	129
P		9933	Kanto	2016	Cat	77	Outdoor	Stray cat	120	106	144	106	115	129
P		9934	Kanto	2016	Cat	78	Outdoor	Stray cat	120	106	144	106	115	129
P		9474	Shikoku	2015	Cat	31	Co-habiting	Co-occurring infection from a cat (No. 34)	120	106	144	106	115	129
P		9497	Shikoku	2015	Cat	34	PS	Purchased at a pet shop	120	106	144	106	115	129
Q	2	9608	Hokuriku	2015	Cat	40	Outdoor	Stray cat	120	108	144	106	115	129
Q		9609	Hokuriku	2015	Human	41	Co-habiting	Owner of a pet cat (No.40)	120	108	144	106	115	129
S	3	8836	Hokuriku	2013	Cat	8	PS	Purchased at a pet shop	120	110	169	106	115	129
S		8877	Hokuriku	2016	Dog	76	PS	Purchased at a pet shop	120	110	169	106	115	129
S		9845	Kanto	2016	Dog	75	PS	Purchased at a pet shop	120	110	169	106	115	129
K1	2	8831†	Hokuriku	2012	Cat	3	Outdoor	Stray cat from the same temple as a cat(No.7)	120	106	144	106	129	129
K1		8835†	Hokuriku	2012	Cat	7	Outdoor	Stray cat from the same temple as a cat(No.3)	120	106	144	106	129	129
K2	3	8903	Shikoku	2013	Cat	9	Outdoor	Stray cat	118	108	114	106	115	129
K2		9783	Shikoku	2016	Cat	72	Outdoor	Household cat going out	118	108	114	106	115	129
K2		9784	Shikoku	2016	Cat	73	Co-habiting	Co-occurring infection from a cat (No. 72)	118	108	114	106	115	129
K3	5	9184	Kanto	2014	Cat	18	Outdoor	Household cat going out	118	106	125	106	115	131
K3		9185	Kanto	2014	Human	19	Co-habiting	Owner of a pet cat (No. 18)	118	106	125	106	115	131
K3		9936	Kanto	2016	Cat	79	Outdoor	Stray cat	118	106	125	106	115	131
K3		10068	Kanto	2016	Cat	90	Outdoor	Stray cat	118	106	125	106	115	131
K3		10069	Kanto	2016	Cat	91	Outdoor	Stray cat	118	106	125	106	115	131
K4	4	9369	Chugoku	2014	Cat	23	Outdoor	Stray cat	118	108	108	106	115	133
K4		9370	Chugoku	2014	Human	24	Co-habiting	Owner of a pet cat (No. 23)	118	108	108	106	115	133
K4		9745	Chugoku	2015	Cat	66	Co-habiting	Infected by a pet owner (No. 67)	118	108	108	106	115	133
K4		9746	Chugoku	2015	Human	67	Others	Owner of a pet cat (No. 66)	118	108	108	106	115	133
K5	2	9607	Kanto	2015	Cat	39	Outdoor	Stray cat	118	106	127	106	115	131
K5		9659	Kanto	2015	Cat	48	Outdoor	Stray cat	118	106	127	106	115	131
K6	1	9635	Kanto	2015	Cat	45	Outdoor	Household cat going out	120	106	146	106	115	129
K7	2	9660	Shikoku	2015	Cat	49	Co-habiting	Co-occurring infection from a cat (No. 60)	118	108	159	106	115	129
K7		9711	Shikoku	2015	Cat	60	Outdoor	Stray cat	118	108	159	106	115	129
K8	2	9663	Hokuriku	2015	Cat	51	PS	After shampooing at a pet shop	120	106	167	106	115	129
K8		9712	Hokuriku	2015	Cat	61	Co-habiting	Co-occurring infection from a cat (No. 51)	120	106	167	106	115	129
K9	3	10042	Hokuriku	2016	Cat	81	Outdoor	Stray cat	120	110	167	106	115	129
K9		10043	Hokuriku	2016	Cat	82	PS	Purchased at a pet shop	120	110	167	106	115	129
K9		10046	Hokuriku	2016	Human	85	Co-habiting	Owner of a pet cat (No. 82)	120	110	167	106	115	129
K10	3	10048	Hokuriku	2016	Cat	87	Outdoor	Stray cat	120	106	163	106	115	129
K10		10049	Hokuriku	2016	Dog	88	Co-habiting	Co-occurring infection from a cat (No. 87)	120	106	163	106	115	129
K10		10050	Hokuriku	2016	Human	89	Co-habiting	Owner of a pet cat (No. 87)	120	106	163	106	115	129
K11	1	10131	Kinki	2017	Cat	92	Outdoor	Stray cat	118	110	169	106	115	129

* Strain number registered with the Department of Dermatology, Kanazawa Medical University

‡ PS:Derived from commercial breeders and pet shops, Outdoor: Stray cats and pet cats that went outdoors and came in contact with stray cats.

Co-habiting: co-occurring infection from the infected animal, Others:Suspected indoor infection or infected by the pet owner.

† There were two genotypes in one strain

‡ After being taken to a family that previously had an infected cat

Table 2. Microsatellite (MS) markers and primer pairs used in this study

MS marker*	Dye		Primer (5' - 3')	Amplicon size(bp) (**)
MS1	6-FAM	F	GAAGGAGGTATATATGGGTGTG	118, 120 (2)
		R	tail-GATAAGGTGTTTGGCACTGA	
MS2	PET	F	GGGAACAATCTGCCTTAAAC	98 to 110 (4)
		R	tail-CACAGAGATATGCCGTATGC	
MS4	NED	F	CAGCATCTAAATAACTGGCCTA	108 to 169 (12)
		R	tail-TTTTCTTTCTACTTCCCGTTG	
MS5	NED	F	GGTTTACACGCAGCATGA	106 (1)
		R	tail-CGTGGCTGAAGAAGTCTACC	
MS6	VIC	F	CGTCTGGGACTTGGTAGTAA	115, 129 (2)
		R	tail-TCGGAGGATCTTTAAACTGT	
MS7	6-FAM	F	GCCAAAGAGCTTGCTGAG	127 to 133 (4)
		R	tail-CGTTAGCATGCATCTCTCTATAC	

* Described by Watanabe et al.[16].

(**)Number of polymorphisms detected.

Table 3. Familial outbreaks of *Microsporium canis* infection

Case No.	KMU No.	Region	Type of secondary infection		Genotype	Route of infection [§]
			Source	Presence of dermatophytosis		
1	8750	Hokuriku	Cat *	positive	P	PS
	8751		Human	positive	P	
2	9171	Kanto	Cat *	positive	P	Outdoor
	9181		Human	positive	P	
3	9184	Kanto	Cat *	positive	K3	Outdoor
	9185		Human	positive	K3	
4	9608	Hokuriku	Cat *	positive	Q	Outdoor
	9609		Human	positive	Q	
5	9369	Chugoku	Cat *	positive	K4	Outdoor
	9370		Human	positive	K4	
6	10043	Hokuriku	Cat *	positive	K9	PS
	10046		Human	positive	K9	
7	9746	Chugoku	Human *	positive	K4	Infected by pet owner
	9745		Cat	positive	K4	
8	9495	Kanto	Cat *	positive	P	Outdoor
	9603		Cat	positive	P	
	9496		Human	positive	P	
9	9632	Hokuriku	Cat *	positive	P	Outdoor
	9634		Cat	negative	P	
	9637		Cat	negative	P	
	9633		Human	negative	P	Human felt itchy
10	10048	Hokuriku	Cat *	positive	K10	Outdoor
	10049		Dog	positive	K10	
	10050		Human	positive	K10	
11	8832	Hokuriku	Dog *	positive	A	PS
	8833		Dog	positive	A	
12	8835	Hokuriku	Cat *	positive	P and K1	Outdoor
	8834		Cat	positive	P	
13	9663	Hokuriku	Cat *	positive	K8	PS
	9712		Cat	positive	K8	
14	9456	Shikoku	Cat**	positive	A	PS
	9457		Cat**	positive	A	
15	9497	Shikoku	Cat *	positive	P	PS
	9474		Cat	positive	P	
16	9711	Shikoku	Cat *	positive	K7	Outdoor
	9660		Cat	positive	K7	
17	9675	Kanto	Cat *	positive	P	Outdoor
	9676~9681		6 cats	all positive	P	
18	9770	Hokuriku	Cat *	positive	I	PS
	9771		Cat	negative	I	
19	9783	Shikoku	Cat *	positive	K2	Outdoor
	9784		Cat	positive	K2	

* Initiator of infection.

** It is unknown which is the initiator because both (Case No. 28 and 29) were purchased together.

[§] PS: Derived from commercial breeders and pet shops, Outdoor: Stray cats and pet cats that went outdoors and came in contact with stray cats.

Table 4. Segregation of genotypes by geographic region

Region	No. of strains	Known genotypes*	New genotypes	No. of each genotype	Route of infection [§]			Co-occurring infection	
					No. of PS	No. of Outdoor	No. of Others	No. of animals	No. of pet owners
Kanto	35	P		21	1	10	0	8	2
			K3	5	0	4	0	0	1
			K5	2	0	2	0	0	0
		A		2	1	1	0	0	0
		F		2	2	0	0	0	0
		G		1	1	0	0	0	0
		S		1	1	0	0	0	0
			K6	1	0	1	0	0	0
Hokuriku	34 [†]	P		11	1	5	0	3	2
		A		5	4	0	0	1	0
		I		3	2	0	0	1	0
			K9	3	1	1	0	0	1
			K10	3	0	1	0	1	1
		L		2	1	1	0	0	0
		Q		2	0	1	0	0	1
		S		2	2	0	0	0	0
			K8	2	1	0	0	1	0
			K1	2	0	2	0	0	0
		D		1	0	0	1	0	0
Shikoku	15	A		4	3 [‡]	0	0	1 [‡]	0
			K2	3	0	2	0	1	0
		L		3	0	3	0	0	0
		P		2	1	0	0	1	0
			K7	2	0	1	0	1	0
		H		1	0	1	0	0	0
Chugoku	8	P		3	1	2	0	0	0
			K4	4	0	1	1	0	2
		B		1	1	0	0	0	0
Kinki	1		K11	1	0	1	0	0	0
All regions	93			95	24	40	2	19	10

* Described by Watanabe et al.[16].

[§] PS:Derived from commercial breeders and pet shops, Outdoor: Stray cats and pet cats that went outdoors and came in contact with stray cats,
Others: Suspected indoor infection or infected by the pet owner.

[†] Among them, 2 strains were considered as a mixture of 2 genotypes ; genotype P and K1.

[‡] Two strains from co-habiting cats (No.28, 29) were separated into origin(PS) and co-occurring infection. It is unknown which was the initiator or co-occurring.

A

B