

**Development and validation of a microsatellite marker-based method for tracing infections by *Microsporum canis*, Journal of Dermatological Science 70 (2) 123-129**

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# Development and validation of a microsatellite marker-based method for tracing infections by *Microsporum canis*

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**Running title**

Use of microsatellite markers for strain typing of *Microsporum canis*

**Key words**

*Microsporum canis*, strain typing, microsatellites, zoonosis, transmission

# Abstract

**Background**

*Microsporum canis* is a dermatophyte fungus harbored by cats and dogs and is frequently transmitted to humans. Molecular tools able to discriminate fungal isolates at the strain level would prove extremely useful for confirming the route of infection, thus contributing to optimization of prophylaxis and hygienic regimens

**Objective**

To develop and validate a microsatellite marker-based method for use in tracking infections by *M. canis*

**Methods**

Primers were designed against sequences flanking the microsatellites individuated by a BLAST search using the nucleotide sequence information assembled by the *Microsporum canis* CBS 113480 genome project. The PCR conditions were standardized and fragment analysis was performed using a genetic analyzer. The resolving power of the markers was investigated on 26 unrelated *M. canis* strains while the reproducibility of the technique and the stability of the markers were evaluated on a single strain subcultured in time as well as on 36 strains isolated from nine outbreak episodes.

**Results**

Eight markers were recognized as being the most polymorphic within the set of *M. canis* strains isolated from unrelated distant hosts, with a total of 22 multilocus genotypes, which corresponded to a genotypic diversity of 97%. Repeated tests on subcultures of *M. canis* reference strain CBS 113480 always yielded the same results. Identical multilocus genotypes were obtained for all the isolates from each outbreak episode.

**Conclusion**

The high resolving power and reproducibility of the markers that were identified support the potential of these tools to detect sources and routes of infection by *M. canis*.

# 1- Introduction

*Microsporum canis* is the zoophilic dermatophyte most commonly harbored by dogs and cats. This fungal species has a worldwide distribution and is frequently implied in episodes of human infection. Indeed, in some countries *M. canis* tends to overpass classical ringworm anthropophilic dermatophytes [1]. Human infections are caused by direct contact with infected animals or, more rarely, with soil or other humans colonized with the fungus [2]. Cats are the most frequent vehicle of the infection, although dogs and occasionally a number of other animal species have been responsible for episodes involving humans [1]. Due to these variable possibilities, tracing the source of infection has proved to be challenging. Yet, the individuation and removal of the source of the fungus is crucial in order to prevent re-contamination of a patient. For this purpose, molecular tools that are able to discriminate fungal isolates at the strain level would be extremely useful for confirming the source of infection. Moreover, such tools would aid in clarifying the transmission dynamics of this fungal pathogen in human and animal populations, thus contributing to optimization of prophylaxis and hygienic regimens [3]. In recent studies, several DNA markers (randomly amplified polymorphic DNA – RAPD - , sequencing of internally transcribed spacer and non-transcribed spacer regions of rRNA genes, intergenic spacers of nuclear DNA, and mitochondrial DNA genes) have been applied to *M. canis*, but there was a low degree of polymorphism within the species [4-8]. One exception was represented by two microsatellite markers developed by Sharma *et al.* (2007) [9]. Microsatellite (MS) DNA sequences are short, tandem-repeating DNA sequences comprised of 1-6 bp per repeating unit. MS are polymorphic in populations due to their propensity for insertion/deletion mutation of multiples of the repeating unit during replication. Variation in the number of repeated units at a genetic

locus is detected by amplifying the alleles by means of the polymerase chain reaction (PCR) using unique primers flanking the repeating sequence, followed by resolving of the PCR products by denaturing electrophoresis [10]. Repeat numbers in alleles are then calculated visually using a sequenced allele with a known repeat number as the reference [9]. Alternatively, primers are labeled with fluorescent dyes and PCR products are loaded onto a genetic analyzer, with results expressed as a colored peak, whose size is calculated by alignment to an internal size standard [11]. Multiple loci are generally used since measures of population structure characteristically show high levels of variance among loci, so that a multilocus genotype is obtained [12]. In other pathogenic fungi, including other dermatophyte species, such as *Trichophyton rubrum* and *Microsporum persicolor*, multilocus microsatellite typing (MLMT) has proved to be a promising tool for uncovering intraspecific diversity [13, 14], and, as anticipated, two microsatellite markers have been already shown to reveal a certain degree of genetic variation in *M. canis* [9]. In the present study, we report on the development of further seven markers, and the analysis of a total of eight microsatellite markers for outbreak typing of *M. canis*.

# 2- Materials and Methods

## 2.1 Experimental design

The markers were initially developed at the Institute of Microbiology and Hygiene, Universitätsmedizin Berlin – Charité, Berlin, Germany (lab A) and subsequently employed and validated with regard to their reproducibility (inter-laboratory reproducibility and *in vitro*/*in vivo* stability of the markers) at the Laboratory of Mycology of the School of Veterinary Medicine of Turin (Italy) (lab B).

## 2.2 Development of the MS markers

### *2.2.1 Fungal strains and DNA extraction*

A total of 26 *M. canis* strains of human and animal origin derived from unrelated locations in 13 countries (Austria, Capo Verde, China, Egypt, France, Germany, Italy, Korea, Mexico, New Zealand, Rep. Dominicana, Turkey, USA) were initially analyzed to individuate the most polymorphic MS markers. Fungal DNA was extracted by the CTAB method [15] after growing the fungus on Sabouraud glucose agar (Difco Laboratories).

### *2.2.2 Design of microsatellite primers*

A BLAST (Basic Local Alignment Search Tool) search using dinucleotide repeat motifs was conducted to identify microsatellite markers by using the nucleotide sequence information assembled by the *Microsporum canis* CBS 113480 genome project (http://www.broadinstitute.org/annotation/genome/dermatophyte_comparative/MultiHome.html). PCR primers between 18 bp and 23 bp in length were designed against sequences flanking the microsatellites detected by the use of Primer3 software [16]. Primers were deduced from sequences 1 to 40 nucleotides upstream and downstream of the microsatellite repeats.

### *2.2.3 PCR and Microsatellite Fragment Analysis*

PCR experiments were performed with fluorescence-conjugated forward primers, by using 6-carboxyfluorescein (6-FAM) and 6 - carboxy - 2',4,4',5',7,7' – exachlorofluorescein (5-HEX) (Sigma-Aldrich Co., St. Louis, MO) as two different labels. The conditions employed for the eight MS markers that were recognized as being the most polymorphic are reported below. PCR assays with all primer pairs (Table 1) were optimized for annealing temperatures and $Mg^{2+}$concentrations; DNA of CBS 113480 and the other *M. canis* strains were used as templates. Finally, each PCR mixture

contained 200 µM of each dNTP, 0.5 U Ampli*Taq* DNA polymerase (Applied Biosystems), and 10 mM Tris-HCl buffer (pH 8.3) containing 50 mM KCl and 1.5 mM $MgCl_2$ (3 for MS1, 3, 4, 5, 6 and 4.5 for MS8), 20 ng template DNA, and 5 pmol of each primer in a final volume of 25 µl. All amplification reactions were performed in a Robocycler Gradient 40 apparatus (Stratagene, La Jolla, CA). After an initial denaturation step of 5 min at 95°C, samples were processed through 35 cycles consisting of 30 s at 95°C, 30 s at the specific annealing temperature indicated in Table 1, and 1 min at 72°C, followed by a terminal elongation step of 6 min at 72°C. The amplified products were commercially analyzed on an automated capillary sequencer (SMB Services in Molecular Biology, Berlin, Germany) using an ABI Prism GeneMapper apparatus (Applied Biosystems, FosterCity, CA).

#### *2.2.4 Data analysis*

The genotype frequencies of each marker were calculated using the software MSA version 3.12 [17], while MULTILOCUS 1.3 (http://www.agapow.net/software/multilocus/) was employed to analyze the genetic diversity of the sample.

## 2.3 Validation of the MS markers

#### *2.3.1 Inter-laboratory reproducibility*

The developed markers were applied to the same fungal strains (26 unrelated *M. canis* isolates from 13 countries) in lab B, with some modifications. Fungal isolates were cultured on Mycobios Selective agar (Biolife Italiana S.r.l., Milan, Italy) and DNA was extracted using a commercially available kit (NucleoSpin® Tissue, Macherey-Nagel, Düren, Germany). Primer sequences (Table 1) were custom synthesized (Applied Biosystems UK) with a fluorescent label attached to the 5' end of each forward primer.

Different dyes (FAM: MS4 and 6; VIC: MS1 and 7; NED: MS2 and 5; PET: MS3 and 8) were employed to allow loading of the PCR products onto the genetic analyzer in two panels, each including four of the MS markers. This avoided confusion due to possible overlapping of allele ranges. Hot-Start *Taq* (Qiagen) (0.5 units) was used, with an initial denaturation for 15 min at 95°C, while the other conditions were unchanged (see section 2.2.3).

Microsatellite fragment analysis was performed using an ABI Prism 310 Genetic Analyzer (Applied Biosystems, FosterCity, CA) for capillary electrophoresis. PCR products were loaded in injections, each including four of the MS markers (panel 1: MS1, 4, 5, 8; panel 2: MS2, 3, 6 and 7). A 1 µL volume of each PCR product was added to 24.0 µL DNA size standard-formamide mix (5 µL LIZ-500 Size Standard and 500 µL Hi- Di formamide, Applied Biosystems) and loaded onto 96-well plates. After capillary electrophoresis, allele calling and analysis were performed using the "microsatellite detector" option and default analysis settings of the GeneMapper version 3.7 software (GeneMapper software version 3.7 User Guide or http://www.appliedbiosystems.com). The software was also used for panel design, allele binning, and data analysis.

***2.3.2 Stability of the MS markers – in vitro and in vivo approach***

Using the modified procedure, the reference strain CBS 113480 was tested on multiple occasions over 6 months of serial passages. Moreover, to evaluate whether the developed markers are stable during *in vivo* transmission of the pathogen, 36 *M. canis* isolates derived from nine episodes of human infection of animal origin were studied. A recently acquired cat was recognized as the source of infection (SOI) in most cases (episode 1, 2, 5, 6, 8, 9), while a recently introduced dog was responsible for infection in episodes 3 and 4. The last case (episode 7) regarded a veterinarian of a zoo-safari

who developed ringworm after having handled 2 infected cheetahs. In some cases more than one human was involved, along with other animals. In such cases the infection may have been spread from the SOI to all the humans/animals living in close proximity, although it cannot be excluded that one of the newly infected subjects infected the others. In some occasions, also environmental isolates were included in the tests. Figures 1 and 2 show the clinical appearance of some of the humans and animals sampled. Some of the *M. canis* strains studied have been deposited into the CBS collection of fungi under accession numbers 124403 (VMT 56), 124406 (VMT 29), 124417 (VMT 59), 124413 (VMT 321) and 124418 (VMT 614). All the other strains are deposited in the fungal collection of the School of Veterinary Medicine of Turin.

# 3. Results

## 3.1 Discriminative power of the method

Of 38 typable MS markers individuated, eight (Table 1) were recognized as the most polymorphic within the set of *M. canis* strains from 26 unrelated distant hosts (Table 2). The remaining 30 markers were unacceptable due to the low variability displayed, i.e. they presented the same size for all the strains or had two alleles but one of them was found only in one strain (data not shown). MS8 had been already developed in an earlier study [9]. The number of alleles revealed by these eight markers ranged from two to 11, with MS6 and MS4 being the least and most polymorphic, respectively (Fig. 3). A single genotype was predominant in six out of eight markers, with a frequency greater than 0.55. The most frequent genotype (MS 6, allele size 107), with a frequency of 0.923 was shared by 24 strains. Analysis of the combined dataset of eight markers detected a total of 22 multilocus genotypes (called A - V) (Table 2), which corresponds to a genotypic diversity of 97%.

### 3.2 Reproducibility of the method

With the modified procedure carried out at lab B on the same dataset of strains, an analogous MS pattern was obtained for all isolates, resulting in 100% inter-laboratory reproducibility. With regard to the stability of the MS markers, the tests repeated on subcultures of *M. canis* reference strain CBS 113480 always yielded the same results (from MS1 to MS 8: 113, 97, 110, 107, 100, 107, 115, 112), while the data regarding the nine episodes of human infection from different localities in Italy are reported in Table 3. The combination of the eight different markers allowed recognition of six multilocus genotypes involved in these episodes. Three genotypes (L, K, and M) have been already discovered within the initial dataset of 26 strains, while the remaining three (W, X, Y) were new (from MS1 to MS8, W = 113, 99, 110, 117, 100, 107, 125, 112; X = 113, 97, 110, 159, 100, 107, 123, 112; Y = 111, 99, 108, 157, 100, 107, 123, 112). Genotypes K and M were responsible for three (ep. 2, 6 and 7) and two (ep. 3 and 5) episodes, respectively, while genotypes L, W, X and Y were involved in one episode each. Importantly, identical multilocus genotypes were obtained for all the isolates from each episode (Table 3).

## 4. Discussion

The genotypic diversity found within the set of 26 unrelated *M. canis* strains (97%) indicates a high resolving power of the markers developed. This MLMT method appears thus to be more promising for the identification of outbreaks and the study of sources of infection by *M. canis* than the markers employed in many of the previous studies, which were instead shown to possess a low discriminatory power [4-8]. With the eight loci studied, the genetic diversity appears to have almost reached a plateau, while with the analysis of only two loci, for example, the diversity was much lower

(71%) [9]. Therefore, in our opinion, scoring more than eight loci would only slightly improve the resolving power of the markers.

Besides the discriminatory power, reproducibility is another key feature required from a strain typing system. Indeed, methods with low or insufficient reproducibility run the risk of incorrectly attributing the presence of multiple genotypes, thus failing to recognize, for example, that a recently acquired animal represents the source of infection within a household. For this reason, in the second part of the study, we focused on the validation of the technique with regard to its reproducibility. This evaluation was not only intended to address the capacity of our method to consistently produce the same results from a single sample, but also the stability of the markers during replication of the fungus. Accordingly, we tested the same strains in two different laboratories and we obtained an identical profile for each sample, regardless of the technical modifications that were introduced to make the procedure less expensive and time-consuming (use of a kit for DNA extraction and of panels including 4 MS each). Afterwards, we obtained the same MS pattern from the same strain (reference strain CBS 113480) after repeated subcultures as well as from the strains involved in each of the nine episodes of human infection of animal origin that were studied. By this approach, we demonstrated the stability of our MS markers during *in vitro* propagation and the *in vivo* transmission of *M. canis*, even in situations with several hosts involved (e.g. episode 6).

Previous studies of the genetic variability of *M. canis* employed prevalently strains coming from epidemiologically unrelated hosts, often from geographically distant locations [4-9], while only a few studies included isolates from closer settings, i.e. isolates sampled from humans or human-animal pairs living in close proximity [2, 18,

19]. Our findings support the fact that testing unrelated isolates is undoubtedly a suitable starting point for revealing the discriminatory power of a method adopted for strain typing: this is because it is more likely that unrelated distant strains show genetic variation. On the other hand, we have also shown that using related isolates makes it possible to ascertain whether the markers developed are sufficiently stable, and the techniques employed are reproducible enough to enable the tracing of a genotype that is being transmitted through various hosts. As previously noted, other studies have tested isolates of the fungus sampled from related hosts [2, 18, 19], but different considerations make it unlikely that the typing methods adopted can represent a mean effective to track *M. canis* infections. For example, in one of these studies [18] sequencing of the ITS region was claimed as a tool usable for cluster analysis and estimation of source of infections by *M. canis*, on the ground that identical ITS1 sequences were found in two fungal strains sampled from a cat and its owner, while differences were noted in sequences of five unrelated strains. However, the authors failed to consider and comment that ITS is the region of choice for species identification and for a basic understanding of phylogenetic relationships among dermatophyte species, but is not suited for applications designed to discriminate between different strains, as only limited sequence variations distinguish closely related species [5, 8, 15]. Moreover, within the same species, sequences are generally highly conserved, and thus probably shared by hundreds of strains. As confirmation, the sequences of the five unrelated isolates presented in the paper [18] show very limited variations.

In other two studies, different molecular markers - ITS sequencing, NTS amplification and RAPD [19], and inter-single-sequence-repeat (ISSR)-PCR [2] – were applied to strains sampled during an outbreak of *tinea capitis* in a school [19] and to strains

coming from some infected patients and their cats [2]. However, these were not infections of known origin usable thus for a validation of the tracking capacity of the markers employed. Indeed, authors could just speculate about the possibility that one [19] or more [2] strains were responsible for the episodes studied. Moreover, some perplexities may arise concerning methods employed and the interpretation of data. For example, as already noted by Abdel-Rahman (2008) [3], the RAPD assay [19] suffered from a significant limitation that appeared to go unrecognized by the authors, namely that the test had probably not been optimized, with non-specific bands obtained on the gels rather than a clean single band. With regard to the other study [2], the stability of the markers employed was not assessed, so that a rapid mutation of the markers cannot be excluded as the cause of the lack of relationship reported for some strains sampled from related hosts. Moreover, the reproducibility of the method was quite low (93%), and based on this value it was arbitrarily assumed that isolates were closely related genetically when the similarity was $\geq$ 93%, which led the authors to deem as identical isolates that did not actually present exactly the same ISSR profile. It is worth underlining that in our research the interpretation was instead very stringent, as we only attributed the same genotype to strains presenting the same allele size at each of the eight loci (Table 3). Moreover, in our study, episodes were only included provided that the humans involved had been healthy before contact with an animal that was recognized as being the source of infection (Table 3). This ruled out cohabiting humans and humans/animals that had acquired the infection from different sources, which in turn allowed us to recognize beyond doubt that one genotype was responsible for each episode, thus indirectly validating the reproducibility of our typing strategy.

The fact that six different multilocus genotypes were individuated from the nine episodes included in our study denotes that a high degree of genetic variability is possible in populations of *M. canis*, even from very close locations (in some cases the same city, see Table 3). This finding suggests that when previous studies demonstrated that several strains from the same locality were found to share the same genotype [6], the reason was probably the low discriminatory power of the markers employed, rather than the possibility that the area sampled was dominated by a single clone. Some caution is warranted however, to this regard, since the epidemiological situation may differ from country to country. For this reason we are currently expanding our dataset of strains to provide an overview of the genetic variability of *M. canis* from different countries in the world, by using our eight MS markers. This will help in interpreting future outbreak episodes, as it must be pointed out that studies that report the same strain among all isolates from a suspected outbreak, occurring in a geographic region for which no baseline data on the degree of variation in the population exists, remain uninterpretable [3].

In conclusion, our results support the usefulness of the MLMT system developed for individuating the source of infection by *M. canis* and clarifying the transmission dynamics of this fungal pathogen among human and animal populations. This method also has the potential to address questions of a different nature; i.e. it may be used to detect markers of virulence and drug resistance in specific genotypes. Indeed, the loci under study are unlikely to be based on these genes, but due to the clonal mode of reproduction of *M. canis*, genomes are transmitted to the next generation in an unaltered condition and thus associated genes – such as virulence genes and microsatellite

markers – are linked, which in turn may facilitate tracing of the feature of interest (virulence, drug resistance etc.) within populations of the fungus [9].

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| Marker | Repeat type | Primer | 5'-3' | bp | $T_a$ (°C) |
|---|---|---|---|---|---|
| MS1 | $GT_{17-1}$ | F | [6-FAM]GAAGGAGGTATATATGGGTGTG | 22 | 54 |
| | | R | GATAAGGTGTTTGGCACTGA | 20 | |
| MS2 | $GT_{17-2}$ | F | [5-HEX]GGGAACAATCTGCCTTAAAC | 20 | 54 |
| | | R | CACAGAGATATGCCGTATGC | 20 | |
| MS3 | $GT_{17-3}$ | F | [5-HEX]AGGTGTTTGGCACTGAGC | 18 | 54 |
| | | R | CGAAGAGAAGGAGGTATATATGG | 23 | |
| MS4 | $GT_{15}$ | F | [6-FAM]CAGCATCTAAATAACTGGCCTA | 22 | 54 |
| | | R | TTTTCTTTCTACTTCCCGTTG | 21 | |
| MS5 | $GT_{14}$ | F | [5-HEX]GGTTTACACGCAGCATGA | 18 | 54 |
| | | R | CGTGGCTGAAGAAGTCTACC | 20 | |
| MS6 | $AT_{15}$ | F | [6-FAM]CGTCTGGGACTTGGTAGTAA | 20 | 58 |
| | | R | TCGGAGGATCTTTAAACTGT | 20 | |
| MS7 | $AC_{20}$-$AT_{14}$ | F | [6-FAM]GCCAAAGAGCTTGCTGAG | 18 | 56 |
| | | R | CGTTAGCATGCATCTCTCTATAC | 23 | |
| MS8 | $GT_{13}$ | F | [6-FAM]GATCGGAGCATGCCATACAG | 20 | 65 |
| | | R | TCTTCCCACCCTTCTCAATG | 20 | |

Table 1. Characteristics of the 8 polymorphic MS markers

F = Forward; R = Reverse; $T_a$ = Annealing temperature

| | | | | Product size (bp) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **source** | **Country** | **City** | **sample** | **MS 1** | **MS 2** | **MS 3** | **MS 4** | **MS 5** | **MS 6** | **MS 7** | **MS 8** | **ML-GT** |
| human | Austria | / | G4 | 113 | 99 | 110 | 117 | 100 | 107 | 123 | 112 | **A** |
| dog | Capo Verde | / | VMT 576 | 113 | 97 | 110 | 121 | 100 | 107 | 123 | 112 | **B** |
| human | China | Hetian | XYZ80032 | 113 | 97 | 110 | 119 | 100 | 107 | 121 | 112 | **C** |
| human | Egypt | Giza | 756 | 113 | 97 | 110 | 157 | 100 | 107 | 121 | 112 | **D** |
| cat | France | Paris | VMT 368 | 113 | 97 | 110 | 117 | 100 | 107 | 125 | 112 | **E** |
| cat | France | Hayange | VMT 374 | 113 | 97 | 110 | 157 | 100 | 107 | 127 | 112 | **F** |
| human | Germany | Würzburg | CBS 113480 | 113 | 97 | 110 | 107 | 100 | 107 | 115 | 112 | **G** |
| human | Germany | Mölbis | 103912/1 | 113 | 97 | 110 | 105 | 100 | 107 | 123 | 112 | **H** |
| human | Germany | Potsdam | 163/8805 | 113 | 89 | 110 | 155 | 100 | 107 | 123 | 112 | **I** |
| cat | Germany | Hamburg | H28 | 113 | 97 | 110 | 157 | 102 | 107 | 123 | 114 | **J** |
| chamois | Italy | Massello | CBS 124423 | 115 | 97 | 112 | 139 | 100 | 107 | 123 | 112 | **K** |
| human | Italy | Firenze | VMT 411 | 115 | 97 | 112 | 139 | 100 | 107 | 123 | 112 | **K** |
| cat | Italy | Cuneo | VMT 114 | 115 | 97 | 112 | 139 | 100 | 107 | 123 | 112 | **K** |
| dog | Italy | Cavallermaggiore | VMT 116 | 115 | 97 | 112 | 139 | 100 | 107 | 123 | 112 | **K** |
| human | Italy | Torino | VMT 186 | 113 | 97 | 110 | 155 | 100 | 107 | 123 | 112 | **L** |
| cat | Italy | Bologna | VMT 323 | 113 | 99 | 110 | 155 | 100 | 107 | 123 | 112 | **M** |
| dog | Italy | Messina | VMT 414 | 113 | 97 | 110 | 119 | 100 | 107 | 125 | 112 | **N** |
| cat | Italy | Padova | VMT 406 | 115 | 97 | 112 | 139 | 100 | 107 | 125 | 112 | **O** |
| cat | Italy | Urbino | VMT 386 | 113 | 97 | 110 | 157 | 100 | 107 | 125 | 112 | **P** |
| dog | Italy | Bergamo | VMT 1 | 113 | 97 | 110 | 157 | 102 | 107 | 125 | 114 | **Q** |
| human | Korea | Kyongki | K9 | 113 | 97 | 110 | 121 | 100 | 107 | 121 | 112 | **R** |
| human | Mexico | Mexico City | Mex12 | 113 | 97 | 110 | 155 | 100 | 107 | 123 | 112 | **L** |
| human | New Zealand | / | CBS 101514 | 109 | 101 | 106 | 149 | 102 | 105 | 121 | 114 | **S** |
| human | Rep. Dominicana | / | Mex10 | 113 | 97 | 110 | 161 | 100 | 107 | 123 | 112 | **T** |
| human | Turkey | Afyon | T2 | 109 | 97 | 106 | 153 | 100 | 107 | 123 | 112 | **U** |
| human | USA | / | CBS 277.62 | 109 | 101 | 106 | 157 | 104 | 105 | 121 | 116 | **V** |

Table 2. Allele sizes (and resulting Multi Locus Genotype) of the eight MS markers from the 26 *M. canis* strains obtained from unrelated hosts. The same sizes were obtained in the two laboratories (lab. A and lab. B) where the study was conducted. MS = Microsatellite; ML-GT = Multi Locus Genotype

| Ep. | sample | | | | |
|---|---|---|---|---|---|
| | **source** | **N°** | **Code** | **ML-GT** | **notes** |
| 1, Biella (Northern Italy) | Cat (SOI) | 1 | VMT 61 | **L** | The human involved was a veterinarian who had kept the cat SOI hospitalized in the clinic for some weeks. During this period some animals (samples 3-5) that were taken to the clinic for other reasons (sterilisation, vaccination etc.) were infected (probably by the vet) |
| | Human | 2 | VMT 613 | **L** | |
| | Cat | 3 | VMT 29 | **L** | |
| | Dwarf Rabbit | 4 | VMT 56 | **L** | |
| | Cat | 5 | VMT 326 | **L** | |
| 2, Turin (Northern Italy) | Cat (SOI) | 6 | VMT 321 | **K** | The patient (sample 7, fig. 1-A) developed ringworm some days after the adoption of a stray kitten with subclinical infection (sample 6, fig. 1-B). Sample 8 was obtained from the cat's couch |
| | Human | 7 | VMT 59 | **K** | |
| | Cat's couch | 8 | VMT 614 | **K** | |
| 3, Turin (School of Veterinary Medicine) (Northern Italy) | Dog (SOI) | 9 | VMT 99 | **M** | The human (sample 10, fig. 1-C) was a veterinary student who handled a dog (sample 9, fig. 1-D) during her clinical training. The fungus was also isolated from the table where the dog had been visited (sample 11) and from another dog that had been hospitalized in the same box (sample 12). |
| | Human | 10 | VMT 117 | **M** | |
| | Table | 11 | VMT 108 | **M** | |
| | Dog | 12 | VMT 107 | **M** | |
| 4, Turin (Northern Italy) | Dog (SOI) | 13 | VMT 135 | **W** | The SOI was a recently acquired dog which infected the new owner (sample 14) and two other dogs already present in the household (samples 15 and 16) |
| | Human | 14 | VMT 146 | **W** | |
| | Dog | 15 | VMT 151 | **W** | |
| | Dog | 16 | VMT 152 | **W** | |
| 5, Villafranca Piemonte (Turin) (Northern Italy) | Cat (SOI) | 17 | VMT 232 | **M** | The SOI was a recently acquired cat which infected the new owner (sample 18) and a cat already present in the household (sample 19) |
| | Human | 18 | VMT 207 | **M** | |
| | Cat | 19 | VMT 233 | **M** | |
| 6, Asti (Northern Italy) and Turin (Northern Italy) | Cat (SOI) | 20 | VMT 341 | **K** | The cat SOI was adopted by a person (sample 21) living in Asti, who developed ringworm on the neck and arms (fig. 2-A). Two dogs of the same household (sample 22 and 23, fig. 2-B) developed lesions as well. The cat was then adopted by a new family and moved to Turin, where it infected other 3 humans (sample 24- 26). Samples 27 and 28 were obtained from environment where these latter patients lived. |
| | Human | 21 | VMT 329 | **K** | |
| | Dog | 22 | VMT 409 | **K** | |
| | Dog | 23 | VMT 410 | **K** | |
| | Human | 24 | VMT 330 | **K** | |
| | Human | 25 | VMT 332 | **K** | |
| | Human | 26 | VMT 333 | **K** | |
| | Sofa | 27 | VMT 384 | **K** | |
| | Floor | 28 | VMT 385 | **K** | |
| 7, Pombia (Northern Italy) | Cheetah (SOI) | 29 | VMT 58 | **K** | The patient (sample 31, fig. 2-C) was a veterinarian of a zoo-safari who developed ringworm after having handled 2 infected cheetahs (fig. 2-D) |
| | Cheetah (SOI) | 30 | VMT 262 | **K** | |
| | Human | 31 | VMT 611 | **K** | |
| 8, Turin (Northern Italy) | Cat (SOI) | 32 | VMT 219 | **X** | The SOI was a recently acquired cat which infected the new owner (sample 33) |
| | Human | 33 | VMT 612 | **X** | |
| 9, Modena (Middle Italy) | Cat (SOI) | 34 | VMT 354 | **Y** | The SOI was a recently acquired cat which infected the new owner (sample 35). The cat was then moved to the house of the owner's mother, who also developed ringworm (sample 36) |
| | Human | 35 | VMT 360 | **Y** | |
| | Human | 36 | VMT 367 | **Y** | |

Table 3. Description of the infection episodes studied by the microsatellite markers. Ep. = Episode number and location. ML-GT = Multi Locus Genotype; SOI = source of infection

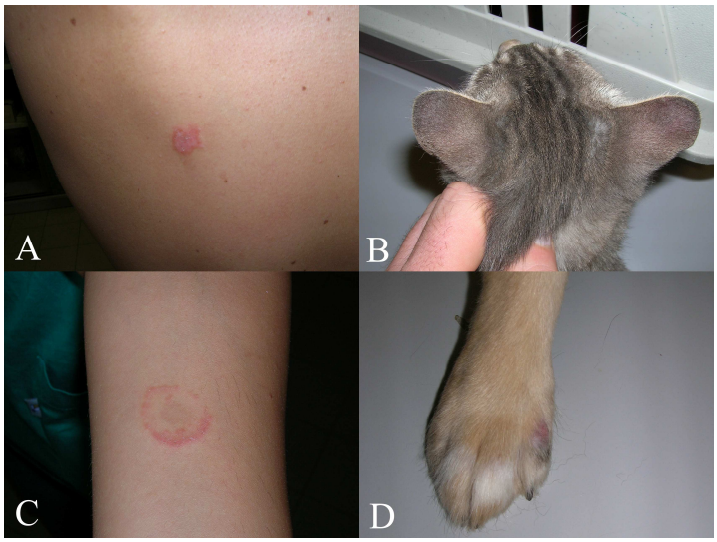


**Fig. 1** Clinical appearance of examples of the humans and animals studied (A and B: sample 7 and 6, episode 2; C and D: sample 10 and 9, episode 3)

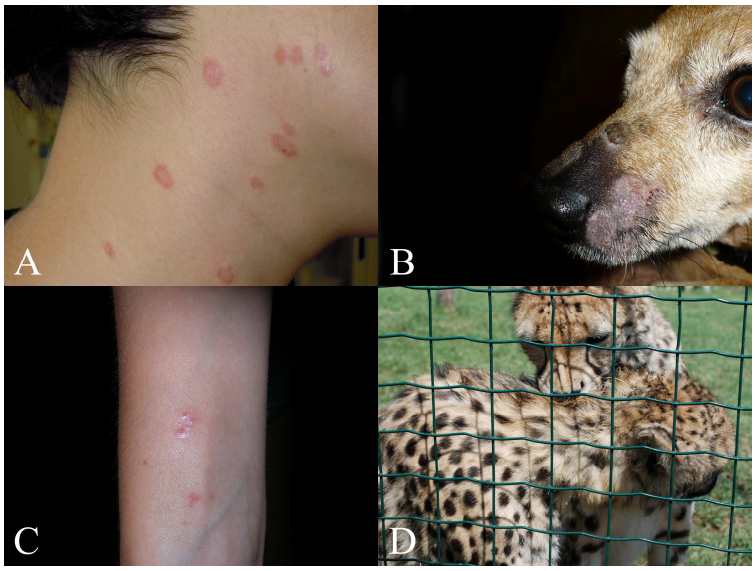


**Fig. 2** Clinical appearance of examples of the humans and animals studied (A and B: sample 21 and 23, episode 6; C and D: sample 31 and 29/30, episode 7)

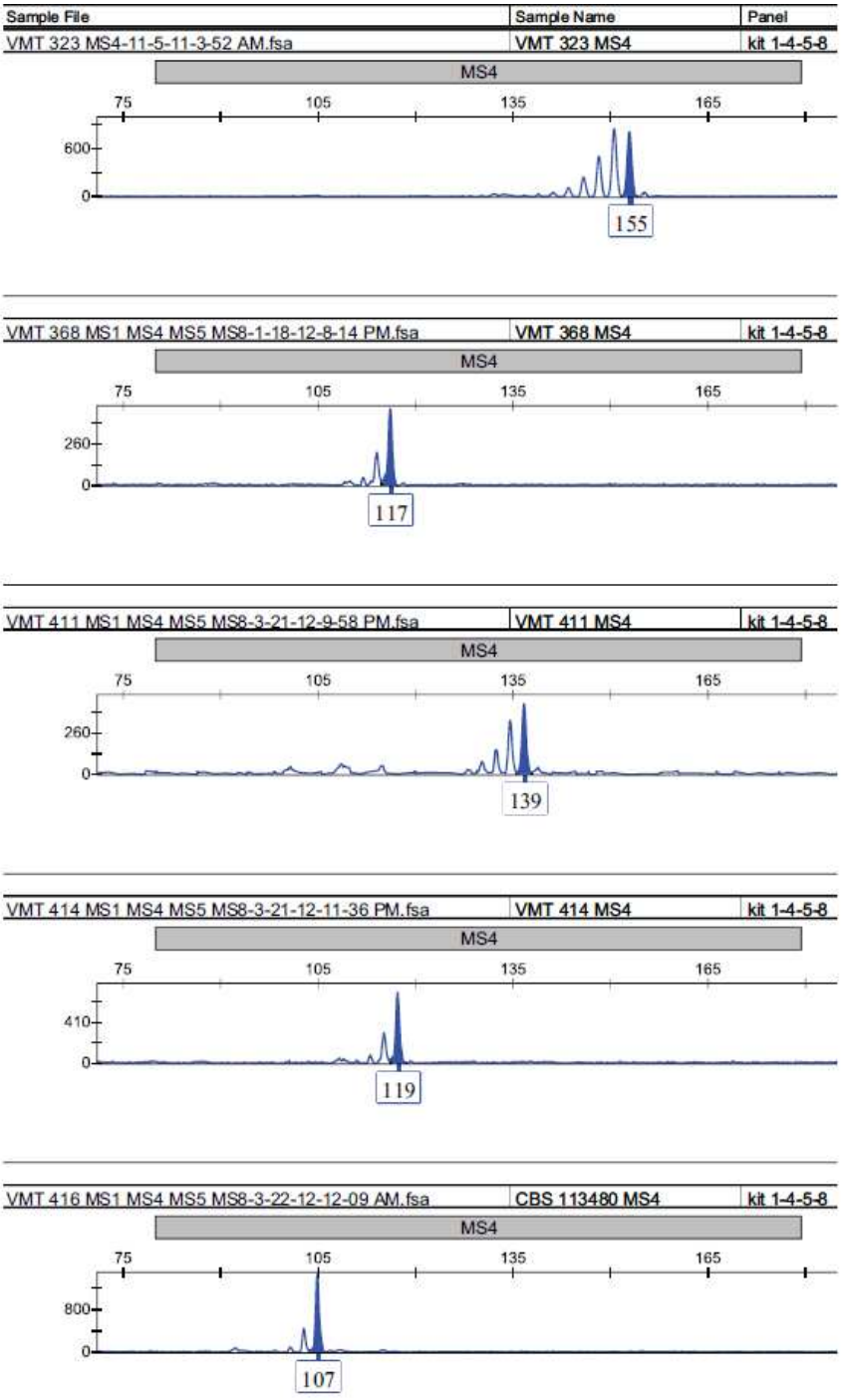


**Fig. 3** Representative image of an ABI GeneMapper file showing the variability of MS4 applied to some unrelated *M. canis* strains. Fragment sizes: 155, 117, 139, 119, 107.