```
VUT-77011 Americano-European race CCCCACGATAGGAATCAACGTTCCATCAGGGGTGTGCAGATGTGCGCCGGCCTTACGCCC  60
RV 30000  African race           ***********G**********************************************
IFM 50998 hedgehogs isolate      ***********G**********************************************

VUT-77011 Americano-European race CATTCTTGTCTACCTTACTCGGTTGCCTCGGCGGGCCGCGCTCTCCTGGGAGAGTCGTCC 120
RV 30000  African race           ************************************************************
IFM 50998 hedgehogs isolate      ************************************************************

VUT-77011 Americano-European race GGCGAGCCTCTTTGGGGGCTTTAGCTGGATCGCGCCCGCCGG_AGGACAGACATCAAAAA 179
RV 30000  African race           ***********C**************************_*********************
IFM 50998 hedgehogs isolate      ***********C**************************T*********************

VUT-77011 Americano-European race ATCTTGGAAAGCTGTCAGTCTGAGCGTTAGCAAGTAAAATT-AGTTAAAACTTTCAACAA 238
RV 30000  African race           ******A********T******************C****-*C*******************
IFM 50998 hedgehogs isolate      ******A********T******************C****-*C*******************

VUT-77011 Americano-European race CGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAAT 298
RV 30000  African race           ************************************************************
IFM 50998 hedgehogs isolate      ************************************************************

VUT-77011 Americano-European race TGCAGAATTCCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCTGGTATTCCGGG 358
RV 30000  African race           ************************************************************
IFM 50998 hedgehogs isolate      ************************************************************

VUT-77011 Americano-European race GGGCATGCCTGTTCGAGCGTCATTTCAACCCCTCAAGCCCGGCTTGTGTGATGGACGACC 418
RV 30000  African race           *********************************C**************************
IFM 50998 hedgehogs isolate      ************************************************************

VUT-77011 Americano-European race GTCCGACCTCCTCTTTCGGGGGCGGGACGCGCCCGAAAAGCAGTGGCCAGGCCGCGATTC 478
RV 30000  African race           *****G*****************T*************************************T
IFM 50998 hedgehogs isolate      *****G*****************T*************************************T

VUT-77011 Americano-European race CGGCTT-CCTGGGCGAATGGGCAGTCAAACCAGCGCCCTCAGGACCGGCCGCTCTGGCCT 537
RV 30000  African race           ******-***************A******C*************************G**
IFM 50998 hedgehogs isolate      ******A***************A******-*************************C**

VUT-77011 Americano-European race TCCCCCAAATCTCTCTGAGATATTTTTTCAGGTTGACCTCGGATCAGGTAGGGATACCCG 597
RV 30000  African race           ********************T***************************************
IFM 50998 hedgehogs isolate      ********************T***************************************

VUT-77011 Americano-European race CT 599
RV 30000  African race           **
IFM 50998 hedgehogs isolate      **
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Fig. 3 Alignments of complete 5.8 rRNA gene and ITS1 and ITS2 region illustrating the sequence divergence among Americano-European race, African race and isolate from hedgehog. Asterisks symbolize identical nucleotides compared to the leader sequence (*A. benhamiae* strain VUT 77011).

was 100% among *CHS1* gene fragments of the five *A. benhamiae* isolates recovered from the hedgehogs and it was almost as high in Americano-European race (97.9%) and African race (98.4%) strains. The sequences reported in this paper have been deposited in the DDBJ database (accession no. AB353723).

The phylogenetic analysis of *CHS1* sequences of the *T. mentagrophytes* complex revealed that they were divided into five clusters. The first cluster consisted of the Americano-European race and eight Japanese isolates from human, rabbits and guinea pig. The second cluster contained the African race isolates, while the third cluster was composed of the five Japanese isolates of *A. benhamiae* from hedgehogs with skin lesions. Finally, the fourth cluster consisted of the *A. simii* strains and the fifth contained the *A. vanbreuseghemii* strains (Fig. 2).

The finding that the five Japanese clinical isolates from hedgehogs were in the same cluster indicated that these strains were genetically closely related to one another and distinct from the Americano-European and African races of *A. benhamiae*.

The results obtained by 5.8 rRNA gene and ITS1 and ITS region analysis (Fig. 3) were phylogenetically similar to that of the *CHS1* analysis (Fig. 4). The nucleotide sequences of the regions of 3 hedgehog isolates and those of hedgehog isolate deposited in database (DDBJ accession no. AB078898) showed 100% similarity.

Nucleotide sequence analysis of the *CHS1* gene fragments from the clinical isolates and standard

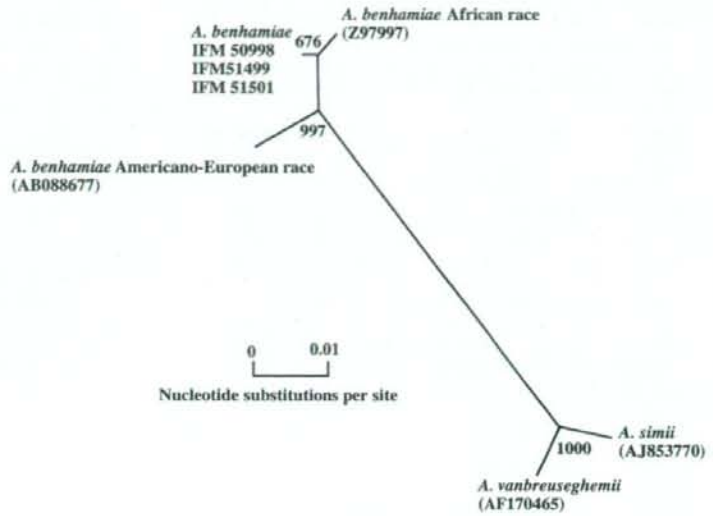

Fig. 4 A tree showing phylogenetic relationships of obtained 5.8 rRNA gene and ITS1 and ITS2 region analysis of dermatophyte species. Numbers at branches were determined by the bootstrap analysis indicating the times in 1000 repeat sub samples in monophylogenic grouping. () indicates DDBJ session number of 5.8 rRNA gene and ITS1 and ITS2 region analysis of dermatophyte species.

strains of *A. benhamiae* (Americano-European race and African race), indicated that the sequence similarities were more than 90% among them (Fig. 1). Therefore, all of these isolates were included in the same species, since the *CHS1* sequence similarities of different species of dermatophytes were at least 10% distant from one another. Moreover, nucleotide sequence analysis of the 5.8 rRNA gene and ITS1 and ITS2 from the clinical isolates and standard strains of *A. benhamiae* (Americano-European race and African race), indicated that the sequence similarities were 97.5–99.3% among them. It has been reported that ITS sequence similarities of different species in dermatophytes were at least 3% distant from one another. The ITS sequence homology among synonymy of geophylic dermatophytes was more than 98% [15]. Therefore, ITS region analysis as *CHS1* sequence analysis in this study indicated that the clinical isolates and standard strains of *A. benhamiae* (both races) might be treated as the same species.

Takahashi et al. reported that the seven clinical isolates from hedgehogs in Japan, five of which were included in this study, showed a distinct mating behavior when compared with the isolates of Americano-European and African races [6]. They implied that these isolates presented a new race [6]. This was confirmed by phylogenetic analysis which revealed that the five Japanese hedgehog isolates were genetically clustered differently from the Americano-European and African races (Figs. 2 and 4). From these results, we propose a new grouping based on the genotypes of *A. benhamiae* on the view points of mating behavior as well as molecular differences; Genotype I consisting of Americano-European race isolates, genotype II containing the African races and finally genotype III composed of our (Japanese) isolates from hedgehogs. We speculated that genotype III may have been transported to Japan from Africa, which is where these hedgehogs (*Atelerix albiventris*) originated. Further molecular investigations on the relatedness of the *A. benhamiae* complex are required to identify the isolates in detail and to know the dissemination route of the genotype III isolates of *A. benhamiae*.

Acknowledgements

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SHORT PAPER

Generalized Hyperkeratosis Caused by *Scopulariopsis brevicaulis* in a Japanese Black Calf

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Summary

A 6-month-old Japanese Black female calf became gradually emaciated over a 40-day period and was humanely killed. At necropsy, hyperkeratotic nodules were seen to have spread over almost the entire body surface. *Scopulariopsis brevicaulis* was isolated from the skin and identified morphologically and by gene sequence analysis of the D1/D2 domain of large subunit ribosomal RNA. Numerous periodic acid-Schiff-positive, lemon-shaped conidia were detected histologically in the keratinized layer and the hair follicles. The distribution of the fungal elements in the skin corresponded to that of the hyperkeratotic lesions. This is the first report of a disease caused by *S. brevicaulis* in animals. Previously reported human infections have not included generalized hyperkeratosis.

Keywords: cattle; dermatitis; fungal infection; generalized hyperkeratosis; *Scopulariopsis brevicaulis*

Scopulariopsis brevicaulis, an annellidic hyphomycete belonging to the phylum *Ascomycota*, is a common soil saprophyte. In human infections it is mainly associated with onychomycosis (nail hyperkeratosis) (Tosti *et al.*, 1996; Goettmann-Bonvallot, 2003; Romano *et al.*, 2005) and keratitis (Malecha, 2004). Cutaneous lesions are rare but include the following: subcutaneous granulomas on the cheek (Bruynzeel and Starink, 1998) and forearm (Sellier *et al.*, 2000); ulcerative granulomatous cheilitis (Creus *et al.*, 1994); neutrophilic follicular inflammation on the legs (Cox and Irving, 1993); and dermal spongiosis on the sole (Ginarte *et al.*, 1996). In addition, disseminated skin lesions were observed in a patient with acquired immunodeficiency syndrome (AIDS) (Dhar and Carey, 1993), but no histological details were provided.

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No histological descriptions of *S. brevicaulis* infections in cattle are available, but the fungus was recovered from 53.3% of bovine claw samples by Abdel-Gawad (1989) and from 1.3% of hair samples by Bagy (1986). Recently it was isolated from abomasal lesions, together with *Cryptosporidium andersoni* (Holko *et al.*, 2004), but the relationship between the fungus and the lesions was not elucidated. The fungus has also been isolated from equine hooves (Keller *et al.*, 2000), buffalo claws (Abdel-Gawad, 1989), canine hair (Bagy, 1986) and ducks claws (Abdel-Gawad and Moharram, 1989). However, its pathogenicity for animals is unknown. This report describes the clinical and histological features of generalized hyperkeratosis associated with *S. brevicaulis* in a calf.

At the end of July 2006, a 4-month-old Japanese Black female calf suddenly developed diarrhoea, depression, anorexia and pyrexia (39.4°C). The animal was treated with sulphonamides, terramycin and gastrointestinal drugs, but its condition did not improve. On August 7, several hyperkeratotic nodules were

detected on the skin of the head, hip and axilla. Despite treatment with ivermectin against parasitic infection, the animal's condition worsened. By August 21, the nodules, showing incrustation, had spread over almost the entire body surface. The animal appeared to be anorexic, inactive and thin, and was treated with ivermectin again. On August 23, the white blood cell count (6200/μl; normal range 4000–12 000/μl) and haematocrit value (43%; normal range 24–46%) were within normal limits. Other values observed were: neutrophils 66% (normal range 17–47%); lymphocytes 34% (normal range 45–75%); monocytes 0% (normal range 2–7%); eosinophils 0% (normal range 2–20%); red blood cell count 14.53×10^6/μl (normal range 5×10^6–10×10^6/μl). Blood chemical analysis revealed elevated values of aspartate aminotransferase (1234 U/litre; normal range 47–75 U/litre), creatine phosphokinase (6780 IU/litre; normal range 66–157 IU/litre), non-esterified fatty acid (1625 μEq/litre; normal range 75–266 μEq/litre), total bilirubin (1.9 mg/dl; normal range 0.1–0.2 mg/dl), blood sugar (63 mg/dl; normal range 43–60 mg/dl) and inorganic phosphate (7.2 mg/dl; normal range 4.8–6.5 mg/dl). On September 6, the animal became emaciated and was humanely killed. No clinical abnormalities were observed in other cattle on the same farm.

At necropsy, the skin nodules with incrustation measured up to 8 mm in diameter. They were generalized in distribution but occurred particularly on the legs (Fig. 1) and head. Muscular oedema and sclerosis were detected in the right hind leg. Numerous abscesses were located in the right anterior lobe of the lung, which was dark red and hard. The parotid and popliteal lymph nodes were enlarged, and the bronchial nodes were enlarged and hard. There were no macroscopical abnormalities in other organs.

The skin of the hind legs was frozen and stored at −80°C for mycological, bacteriological and virological studies.

For mycological study, small pieces (5–7 mm^3) of the skin, liver, spleen, heart, parotid lymph node and brain were cultured at 30°C for 7 days on the following media: potato dextrose agar (PDA) containing chloramphenicol 100 mg/litre; brain heart infusion agar containing yeast extract 1%, dextrose 1% and chloramphenicol 100 mg/litre; and CHROMagar™ Candida (Kanto Regent, Tokyo). The colonies that grew from skin samples were composed of fungal mycelia and produced a colour reaction on CHROMagar™ Candida. The skin isolate was subcultured on PDA and Sabouraud glucose agar (SDA) at 25°C for 10 days. The isolate grown on PDA was also examined microscopically after mounting in lactophenol cotton blue.

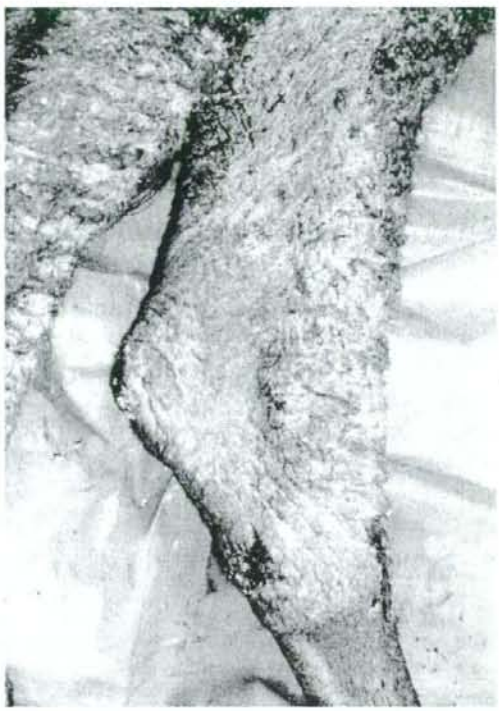

Fig. 1. Hyperkeratosis in the right hind leg.

The D1/D2 domain of the large subunit ribosomal RNA gene (D1/D2 LSU) sequence for the fungus isolated from the skin was processed by a standard method (Kurtzman and Robnett, 1997). DNA was extracted from a PDA slant culture grown at 25°C for 5 days. The sequence of the isolate was compared *via* a basic local alignment search tool (BLAST) search in the GenBank data base, and its location was determined by a distance tree of the results.

For bacteriological study, the samples (skin, liver, spleen, kidneys, heart, lung and brain) were cultured on 5% sheep blood agar and on desoxycholate hydrogen sulphide lactose agar (DHL; Nissui Pharmaceutical Co., Tokyo, Japan). The plates were incubated for 48 h at 37°C aerobically, microaerobically (carbon dioxide 10%, oxygen 5% and nitrogen 85%) and anaerobically. The isolates were identified with commercial identification kits (API Staph, Api-Coryne; bioMérieux Japan, Tokyo, Japan).

No specific genes for parapoxvirus or bovine viral diarrhoea virus were detected in skin samples examined by the polymerase chain reaction (PCR). No pathogenic virus was isolated from the skin in several cell line cultures that included Madin-Darby bovine kidney (MDBK), bovine kidney (BK) and bovine

fetal muscle (BFM) cells. The presence of antibodies to bovine leukaemia virus (BLV) was excluded by the agar gel immunodiffusion test and BLV antigen kit "Hokken" (Kitasato Institute Research Center for Biologicals, Japan).

For histological examination, samples of skin (legs and head), lymph nodes (popliteal and mesenteric), lung, liver, spleen, kidney, heart, tongue, oesophagus, stomach, intestines (small and large), muscle and brain were collected within 30 min of death. They were fixed in 20% phosphate-buffered formalin and embedded in paraffin wax. Tissue sections (3 μm) were stained with haematoxylin and eosin (HE). In addition, skin and lung sections were stained with Gram, Grocott, periodic acid-Schiff (PAS), Warthin–Starry and Giemsa stains.

Serial skin sections were prepared for immunohistochemical labelling by the Universal Immunoenzyme Polymer method, with a Histofine Simple Stain MAX-PO Kit (Nichirei Corp., Tokyo, Japan). The sections were pre-treated with 0.1% trypsin, and endogenous peroxidase activity was blocked by H_2O_2 3% in methanol.

The primary antibodies used were as follows: mouse monoclonal antibodies to *Aspergillus fumigatus* wall fractions (M3564; Dako, Carpinteria, CA, USA) and to water-soluble somatic antigens from *Rhizopus arrhizus* (M3565; Dako); and rabbit polyclonal antibodies to *Candida albicans* (1750-5507; Biogenesis, Poole Dorset, UK), bovine papilloma virus (Quartett, Berlin, Germany) and *Arcanobacterium pyogenes* (Ohba *et al.*, 2007). Sections were lightly counterstained with haematoxylin. Simultaneously, bovine or human tissues infected with *Aspergillus*, *Zygomycetes*, *Candida*, *A. pyogenes* or papilloma virus were immunolabelled as positive controls (Yokota *et al.*, 2004a,b; Maeda *et al.*, 2007; Ohba *et al.*, 2007). Negative controls were prepared by replacing the primary antibody with phosphate-buffered saline or non-immune mouse or rabbit serum.

Mycological cultures from skin lesions on PDA or SDA showed characteristic colonies of *S. brevicaulis*. They were greyish-white at first, later becoming powdery and light brown centrally, with a white periphery. Microscopically, annellidic conidiogenous cells were detected, the conidia being round or lemon-shaped, with a truncated base or with a short neck at the basal edge. The mature conidia, approximately 7–8 μm in diameter, were rough (spiny or warty) and non-pigmented (Fig. 2). The hyphae were septate and non-pigmented. The characteristic morphology of the conidia and conidiophores was consistent with *S. brevicaulis*.

One of the isolates that grew on the PDA plate was designated as SO28781. The D1/D2 LSU sequence

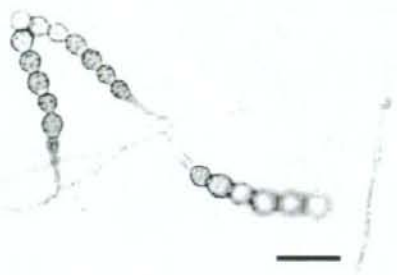

Fig. 2. Annelloconidia of isolate SO28781. The conidia are round or lemon-shaped, with a truncated base or with a short neck at the basal edge. The mature conidia are rough and spiny. Lactophenol cotton blue staining. Bar, 20 μm.

consisted of 608 base pairs designated as AB297478. This showed that isolate SO28781 was located at the cluster consisting of the sequences derived from *S. brevicaulis*, with an identity of 99% in the D2 domain of the LSU sequence consisting of 314 base pairs by a draft distance tree of the results. Isolate SO28781 was therefore identified as *S. brevicaulis*.

Several fungal species (e.g., *Penicillium* sp., *Aspergillus flavus*, *Paecilomyces lilacinus* and *Candida glabrata*) were isolated from sites other than the skin, but were considered to be environmental contaminants because of their absence in the histological sections. *Staphylococcus* spp. and *A. pyogenes* were isolated from the skin and lung, respectively.

Histopathological examination of the skin lesions revealed marked hyperkeratosis and parakeratosis with an overlying crust (Fig. 3), numerous PAS-positive fungi and a moderate number of gram-positive bacteria. The fungi were present chiefly in the keratinized layer and hair follicles (Fig. 4). Hyphal elements were sparse, but lemon-shaped, thick-walled spores were abundant (Fig. 5). The bacteria were randomly distributed in the keratinized layer in some of the lesions. The underlying dermis, which was markedly oedematous, was infiltrated by numerous lymphocytes and plasma cells. There was no evidence of intranuclear or intracytoplasmic viral inclusions.

Severe suppurative bronchopneumonia was seen in the lung, and multifocal necrosis with degenerating neutrophils and fibrin deposits was observed. Gram-positive bacilli were detected in the lesions, but Grocott staining and PAS staining for fungi were negative.

Other abnormalities included marked depletion of lymphocytes in the systemic lymphoid organs, moderate interstitial nephritis, mild centrilobular

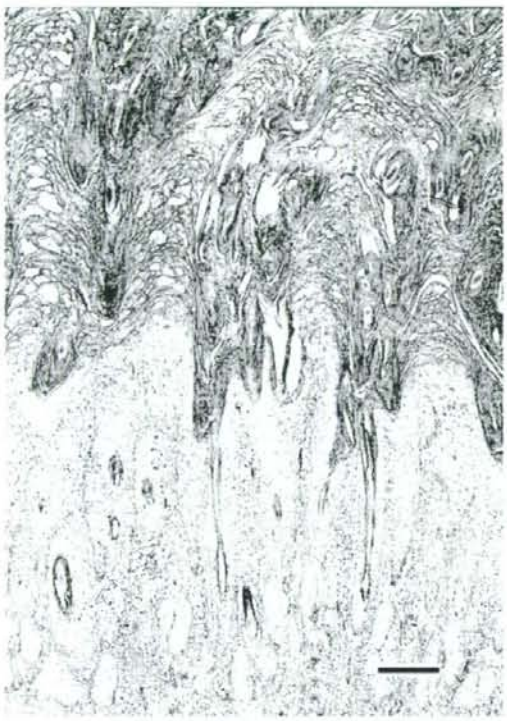

Fig. 3. Skin. Extensive areas of hyperkeratosis and parakeratosis are visible. HE. Bar, 250 μm.

hepatocellular vacuolar degeneration, mild non-suppurative myocarditis, and mild suppurative glossitis. In addition, necrotic myositis was seen in the right hind leg. These changes were not associated with the presence of pathogenic organisms.

Immunohistochemistry failed to reveal any evidence of other infectious agents in the skin. In the lung, *A. pyogenes* antigen was detected in the suppurative lesions, its distribution corresponding to that of gram-positive bacteria.

The cutaneous disease reported here was judged to be generalized hyperkeratosis caused by *S. brevicaulis*, on the basis of the mycological and histological findings. This is the first reported case of a disease caused by *S. brevicaulis* in animals; moreover, generalized hyperkeratosis caused by *S. brevicaulis* has not been reported in man. *Staphylococcus* spp., also isolated from the skin, were not associated with the hyperkeratosis because they were seen in only some of the lesions. Persistent bovine dermatomycosis (ringworm), which is exclusively caused by *Trichophyton verrucosum* (Wabacha *et al.*, 1998), is characterized by annular skin lesions, with alopecia. Like *S. brevicaulis*, the

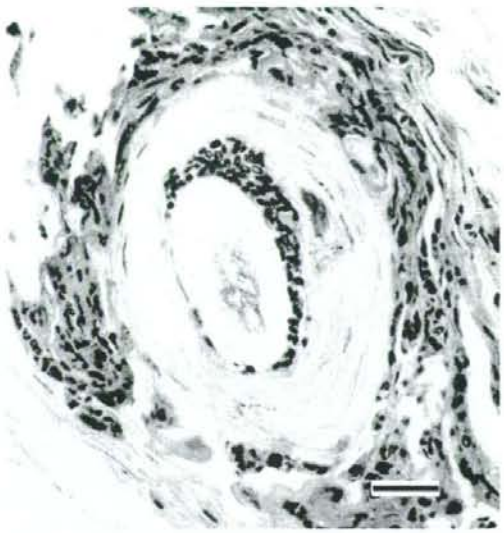

Fig. 4. Skin. Numerous PAS-positive conidia are present in a hair follicle. PAS. Bar, 20 μm.

dermatophyte affects the keratin layer of the skin; it is characterized, however, by hyphae that may fragment into arthrospores (Wabacha *et al.*, 1998). Thus, these two invasive fungi are readily distinguishable.

Human cutaneous *S. brevicaulis* infection has been reported in a few non-immunocompromised patients (Cox and Irving, 1993; Creus *et al.*, 1994; Ginarte *et al.*, 1996; Bruynzeel and Starink, 1998), in an elderly man with a liver transplant (Sellier *et al.*, 2000) and in an AIDS patient (Dhar and Carey, 1993). The area of the skin lesions in the AIDS patient was much greater than in the other patients, and it

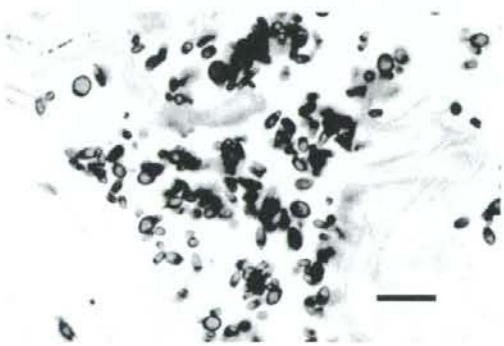

Fig. 5. Skin. Numerous lemon-shaped, thick-walled spores are visible in the keratinized layer. Grocott. Bar, 10 μm.

would appear that predisposing factors for the fungal infection include AIDS, corticosteroid therapy and organ transplantation (Phillips *et al.*, 1989; Dhar and Carey, 1993; Sellier *et al.*, 2000). In the calf described in the present report, the cutaneous lesions were generalized and thus distinct from the localized ones found in non-immunocompromised human patients. In view of the clinical findings (anorexia and emaciation) and pathological findings (marked depletion of lymphocytes), the calf may also have suffered from an immunosuppressive disease; this might have increased its susceptibility to the fungal dermatitis and to the pneumonia associated with *A. pyogenes*, a known opportunistic pathogen (Jost and Billington, 2005).

Bovine leucocyte adhesion deficiency (BLAD) in Holstein cattle is an autosomal recessive congenital disease characterized by recurrent bacterial infections, delayed wound healing and stunted growth, and is also associated with persistent marked neutrophilia (Nagahata, 2004). It is unclear whether BLAD occurs in the Japanese Black breed. Some of the clinical features (e.g., neutrophilia and glossitis) described here were similar to those of BLAD cases (Nagahata, 2004). However, severe ulcers on oral mucous membrane, ulcerative stomatitis, gingivitis and loss of teeth were not seen; moreover, numerous neutrophils were detected in lesions of suppurative pneumonia. The case described thus differed from BLAD.

It is difficult to identify the source and route of *S. brevicaulis* infection even in human cases. In a 14-year-old girl, the fungal lesion apparently developed at the site of an abrasion which became infected from clothing usually worn while riding and caring for a horse (Cox and Irving, 1993); the source of the contamination was suspected to be equine feed or bedding. It is possible that an abrasion and contaminated feed or bedding were associated with the case described in this report.

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短　報

漢方生薬配合薬の抗真菌活性と牛白癬の治療効果

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要　約

漢方生薬配合薬の抗真菌活性を検討したところ，本剤は多くの真菌に対して発育を抑制した．管内肥育牧場で発生した牛白癬3例に応用した結果，本剤の1週間経口投与と漢方生薬10％煎じ液の4日間体表噴霧の併用治療は著効を示した．――キーワード：抗真菌活性，漢方生薬，牛白癬．

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牛皮膚糸状菌症（牛白癬）は，牛だけでなく人への感染も起こす人と動物の共通感染症の一つであり，そのおもな原因菌は*Trichophyton verrucosum*（以下*T. verrucosum*）である［1］．本症は全国的に発生が報告されており［2，3］，感染性が非常に強く，集団飼育された牛群で発生が多くみられ，発症すると増体に影響を及ぼし経済的損失を招くことが報告されている［3］．本症の治療には，抗真菌剤や消毒薬が有効とされており，経済性と治療効果の確実性および使用の簡便性などが要求されるが，それらの条件に適した薬剤は乏しいのが現状である［4］．

漢方生薬配合薬は，本来動物の消化器疾患治療薬として認可されているが，本剤にはオウバク末，オウゴン末など抗真菌活性を示す成分［5］が含まれている．そこで今回，本剤の抗真菌活性と*T. verrucosum*に感染した牛白癬への治療効果を調査した．

材料および方法

漢方生薬配合薬の抗真菌活性の調査は，2005年3月から2005年5月の間に千葉大学真菌医学研究センターにて保存している人と動物の共通感染症原因真菌9株を用いて，漢方生薬配合薬（新中森獣医散®，中森製薬㈱，宮崎）を0.1％，0.3％，1.0％，3.0％，10％添加して121℃20分オートクレーブ滅菌して作成した1/10サブロー寒天（0.2％グルコース，0.1％ペプトン，1.5％寒天）に各濃度3枚ずつ1白金耳接種した．培養温度は35℃で，培養時間は2～4週間（菌種により異なる）とし，培養後のコロニーの直径を計測した．

牛白癬への治療効果の調査は，管内A肥育牧場（哺育牛導入，肥育素牛販売，300頭飼養）で，2005年6月から2005年11月に*T. verrucosum*が検出された牛白癬症例3頭について実施した．治療方法は，本剤0.05～0.2g/kgの1週間飼料添加と漢方生薬10％煎じ液の連続4日間体表噴霧を行った．なお，漢方生薬10％煎じ液は水分が半分になるまで煮つめて濾過し，皮膚浸透性を高めるためにDimethyl sulfoxide（DMSO）を1％添加して作成した．効果は，治療前，治療最終日，治療終了翌日とその後1週間ごとの患部の観察と患部から採取した鱗屑1白金耳あたりの真菌の培養コロニー数で判定した．また，培養コロニー数カウントの上限は50までとした．

成　績

表1に本剤の各共通感染症原因真菌に対する抗真菌活性の結果を示した．おもに牛・馬に発症する*T. verrucosum*は，漢方生薬濃度0.3％から発育抑制が始まり10％で発育阻止がみられた．また，その他の真菌においても10％で発育阻止がみられた．

表2に牛白癬症例3頭の体表に発現した患部における真菌コロニー数の経時的推移を示した．症例1では真菌コロニー数が治療後1週目から減少し，7週目で急激に発毛が始まり，治療後9週目で完治した（図1）．症例2では，真菌コロニー数が治療後2週目からほぼ消失した．また，治療後5週目で急激に発毛し，治療後7週目で完治した．

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表1　漢方生薬配合薬濃度別にみた原因真菌コロニーの直径（cm）

菌名	菌株番号	漢方生薬配合薬濃度（%）					
		0	0.1	0.3	1.0	3.0	10.0
Trichophyton verrucosum	IFM 46012	3.5	2.8	2.7	0.2	0.2	0
		(3.4–3.6)	(2.8)	(2.5–2.8)	(0–0.7)	(0–0.4)	0
		100	80.0	77.1	5.7	5.7	0
T. verrucosum	IFM 46798	1.9	2.7	0.5	0.3	0.1	0
		(1.8–2.0)	(2.6–2.8)	(0–1.6)	(0–0.9)	(0–0.1)	0
		100	142	26.3	15.8	5.3	0
T. mentagrophytes	IFM 53814	3.3	3.5	3.4	2.5	2.4	0
		(3.2–3.4)	(3.0–4.2)	(3.0–4.2)	(2.2–2.8)	(2.0–2.8)	0
		100	106	103	75.8	72.7	0
T. mentagrophytes var. *erinacei*	IFM 50998	6.6	7.2	6.7	6.7	5.9	2.7
		(6.2–6.8)	(7.0–7.4)	(6.4–6.8)	(6.6–6.8)	(5.8–6.0)	(2.6–2.8)
		100	109	102	102	89.4	40.9
T. rubrum	IFM 53813	6.0	5.2	0	0	0	0
		(5.8–6.2)	(4.7–5.7)	0	0	0	0
		100	86.7	0	0	0	0
T. tonsurans	IFM 52825	4.5	4.0	3.4	2.1	2.1	0
		(4.1–4.9)	(3.7–4.2)	(2.8–3.9)	(1.8–2.4)	(2.0–2.2)	0
		100	88.9	75.6	35.0	35.0	0
Microsporum canis	IFM 53931	6.6	6.4	5.6	4.4	0	0
		(6.5–6.8)	(6.4–6.5)	(5.3–6.0)	(4.4)	0	0
		100	97.0	84.8	66.7	0	0
M. canis	IFM 54149	5.7	5.7	5.2	0	0	0
		(5.5–5.8)	(5.5–5.8)	(4.5–5.3)	0	0	0
		100	100	91.2	0	0	0
M. gypseum	IFM 53792	7.3	7.1	6.0	3.0	1.6	0.5
		(7.2–7.4)	(7.0–7.2)	(5.8–6.2)	(2.8–3.2)	(1.4–1.8)	(0–0.8)
		100	97.3	82.2	41.1	21.9	6.8

注：最上段は3枚の培地の平均値を示す．最下段は本剤を添加しないときの発育直径を100%としたときの%を示す．

表2　症例の真菌コロニー数の推移

採材日	供試牛		
	症例1	症例2	症例3
治療前	>50	>50	>50
治療最終日	>50	>50	>50
治療終了翌日	>50	>50	>50
1週目	28	>50	>50
2週目	46	0	7
3週目	21	7	>50
4週目	2	7	>50
5週目	10	0	>50
6週目	3	1	39
7週目	4	治癒	>50（再治療）
8週目	2		>50
9週目	治癒		7
10週目			20
11週目			1
12週目			4
13週目			1
14週目			4
15週目			7
16週目			4
20週目			治癒

治療効果出現が遅延した症例3では，治療後3週目から真菌コロニー数の上昇を示し，治療後5週目を経過しても体毛再生がみられなかった．そこで，治療後7週目に再度，漢方生薬10%煎じ液の4日間連続体表噴霧のみを行ったところ，治療後9週目から真菌コロニー数の減少がみられ，体毛再生も始まり，治療後20週目で完治した．

考　察

今回の調査により，漢方生薬配合薬は，人と動物の共通感染症の原因菌である多くの真菌に対して発育を抑制することが明らかになった．特に，人の水虫の原因菌とされる*T. rubrum* [6] に対しては，漢方生薬濃度0.3%以上で発育阻止を示し，強い抗真菌活性がみられた．また，菌種によって感受性は異なるものの，多くの真菌において漢方生薬濃度10%で発育阻止を示すことが明らかになった．

牛白癬は，若齢牛や栄養不良の老齢牛に好発し，牛どうしの接触や作業器具などから間接的に感染し，春から夏にかけて多発するといわれている [7]．その主要な原

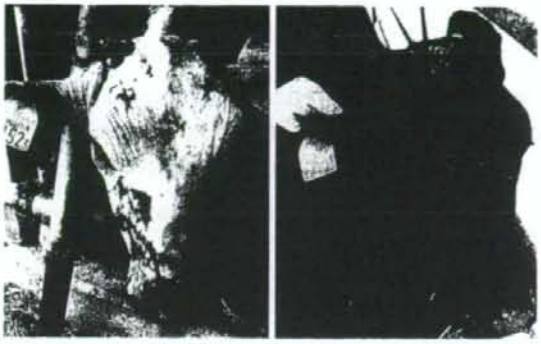

図1　症例1の治癒経過（左は治療前，右は治療後9週目）

因菌は*T. verrucosum*である［1］．今回，*T. verrucosum*に感染した牛白癬症例に対して，本剤の経口投与と煎じ液の体表噴霧の併用治療は，有効であった．症例1，2においては，治癒に至るまで約2カ月間を要し，塩化ジデシルジメチルアンモニウム溶液を用いて治療した報告［2, 3］と同等の治療期間であった．また，真菌コロニー数の減少に対して体毛再生の開始は数週間遅延して生じることが明らかになった．いっぽう，治療効果出現が遅延した症例3では，治療後3週目から真菌コロニー数の増加がみられたが，その原因として，接触による再感染が考えられた．したがって，4日間連続体表噴霧後5週目を目安に症状の改善がみられない場合は，本剤の再噴霧が必要であると考えられた．

以上のことから，牛白癬に対し漢方生薬配合薬を，10％煎じ液として病変部に直接噴霧し，経口投与することが有効であることが分かった．今回の試験では，煎じ液の体表噴霧のみの効果について調査しなかったが，症例3の再治療で体表噴霧のみで完治したことから，今回の治療効果の主要部分は煎じ液を外用で用いたことによると考えられた．

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Anti-Fungal Effect of Chinese Herbal Combination Drug and Efficacy with Cow Ringworm

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SUMMARY

The objective was to examine the anti-fungal effects of a Chinese herbal combination drug. The results demonstrated that this drug was effective in controlling many fungus growths. The drug was applied to three calves with cow ringworm on a local fattening ranch. The combined treatment of external applications of a 10% Chinese herbal infusion liquid solution over four days and internal administration of the Chinese herbal drug for one week showed remarkable results. —— Key words : Anti-fungal, Chinese herbal medicine, cow ringworm.

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Short Communication

Epidemiology of Sporadic (Non-Epidemic) Cases of *Trichophyton tonsurans* Infection in Japan Based on PCR-RFLP Analysis of Non-Transcribed Spacer Region of Ribosomal RNA Gene

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SUMMARY: A number of cases of *Trichophyton tonsurans* infection have been reported among sportsmen and women participating in wrestling, judo, and sumo wrestling in Japan, but there have also been sporadic reports of cases with no history of contact with these sports. A molecular method using restriction enzyme analysis of PCR-amplified fragments targeting the non-transcribed spacer region (NTS) of ribosomal RNA gene in fungal nuclei was applied to *T. tonsurans* strains isolated from sporadic cases in Japan. Five of 6 molecular types recorded in Japan, i.e., NTS types I, II, IV, V, and VI, and two new types, designated NTS VII and NTS VIII, were observed among 10 strains isolated from sporadic cases. The NTS IV strains, considered not to be related to the present epidemic, were found to be the most prevalent molecular type accounting for 4 of the 10 strains isolated. NTS I was the most prevalent type in the current epidemic in Japan, but it was cultured from only one patient who was later noted to be the daughter of a retired judo practitioner. Four subjects had histories of living abroad and were considered to have been infected outside Japan. The strains in these cases were NTS II, V, VI, and VII. The results of this study suggested that the NTS IV strains were originally present in Japan at a low incidence, but that there has been a recent influx of NTS I, II, V, VI, and VII from abroad, which has been accompanied by the secondary spread of strains from wrestlers and practitioners of martial arts to the general community.

Trichophyton tonsurans is known to be the causative agent of tinea capitis and tinea corporis worldwide. Since 2001, a nationwide epidemic of *T. tonsurans* infection has been documented among sportsmen and women participating in wrestling, judo, and sumo wrestling in Japan (1-3). Before the epidemic, *T. tonsurans* had been reported to be responsible for several sporadic and familial cases of tinea capitis in Japan (4-7). In a previous molecular epidemiological study using restriction fragment length polymorphism (RFLP) analysis of the non-transcribed spacer region (NTS) of ribosomal RNA gene with the restriction enzymes *Mva*I and *Ava*I (8), we distinguished 6 molecular types among Japanese isolates: NTS I, II, III, IV, V, and VI. Among these types, the epidemic was predominantly caused by NTS I isolates, whereas 7 sporadic cases were caused by NTS II (2 cases), IV (3 cases), V (1 case), and VI (1 case). These findings suggested that the epidemic was not derived from these sporadic cases but originated outside Japan through international sports activity and has spread secondarily in Japan (2,8). Analysis of intraspecies polymorphisms of the strain is thus important for epidemiology, and may be useful for infection control. The present study was performed to evaluate the source of infection of sporadic cases of *T. tonsurans* infection.

Ten strains (7 strains described above and 3 newly isolated strains) of *T. tonsurans* isolated from sporadic cases, i.e., patients considered not to be engaged in contact sports or to be family members or intimate relations of contact sports players at the initial visit to regional dermatologists, were selected from about 240 strains in our culture collection (Table 1). Four of the ten strains were related to travel abroad, two isolated from immigrants and the other two were from patients who had remained abroad for long periods.

To identify the strains as *T. tonsurans*, we first observed the gross features of their colony morphology, and then confirmed that the strains were compatible with *T. tonsurans* by PCR-RFLP analysis of the ITS regions of the rRNA genes (9). The method of analysis for the NTS regions was described previously (8).

Briefly, total cellular DNA was extracted from colonies cultured on Sabouraud's dextrose agar slants or plates with or without antibiotics by a rapid preparation method (10) and used as the template for PCR. To confirm identification of the strains as *T. tonsurans*, universal primers targeting the ITS regions, i.e., ITS1 (5'-TCCGTAGGTGAACCTGCGG) and ITS4 (5'-TCCTCCGCTTATTGATATGC) (11), were used. The thermal cycler was programed for 4 min at 94°C followed by 35 cycles of 1 min at 94°C, 2 min at 58°C, and 1.5 min at 72°C. The amplicon was digested with the restriction enzymes *Mva*I (Toyobo Co. Ltd., Osaka, Japan) and *Hin*fI (Toyobo), and then electrophoresed on 5% polyacrylamide gels, stained with ethidium bromide, and observed under a UV lamp (8). For observation of the NTS regions, PCR amplicons generated using the templates and the primer pair, L663 (5'-TTCTAGGCTCCCAACCAC) and R1145 (5'-ACA AGGGCGGAACTATCAGAC) targeting the middle part of

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Table 1. *Trichophyton tonsurans* strains used in the study and their molecular types

Case	Patient	Lesion	Year /place of isolation	Reference	KMU#	Molecular type	Remarks
1	62 F	Tinea capitis (BDR)	1989 Niigata	Oka and Shimizu (6)	3188	NTS IV	
2	65 F	Tinea capitis (BDR)	1990 Akita	Sato et al. (7)	3313	NTS IV	
3	74 F	Tinea capitis (BDR)	2001 Niigata	Fujita et al. (12)	4251	NTS IV	
4	10 F	Tinea capitis	2001 Shizuoka	Urano et al. (15)	4253	NTS VI	Immigrant from Peru
5	8 F	Kerion	2001 Gifu	Tosaki and Fujihiro (19)	4254	NTS VIII	
6	6 M	Tinea capitis	2002 Tokyo	Kitami et al. (14)	4409	NTS II	Lived in Singapore
7	11 M	Tinea capitis	2005 Kyoto	Arakawa et al. (16)	4855	NTS V	Lived in USA
8	0 F	Tinea capitis	2006 Osaka	Sasagawa (20)	4918	NTS I	Mother: tinea capitis
9	74 F	Tinea capitis (BDR)	2002 Chiba	Takahashi et al. (13)	6028	NTS IV	Recurrence after treatment
10	17 F	Tinea capitis	2007 Shizuoka	Urano et al. (17)	6099	NTS VII	Immigrant from Peru

BDR, black dot ringworm.

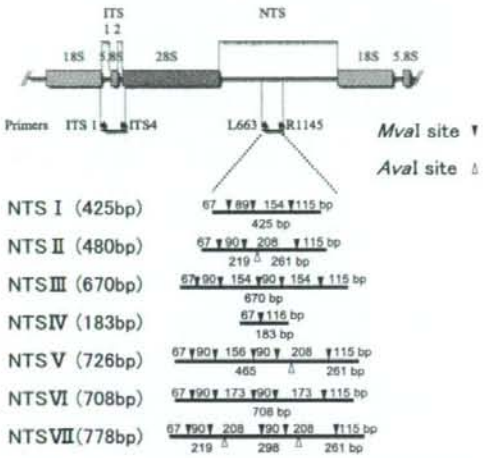

Fig. 1. NTS region of ribosomal RNA gene and restriction maps for each of the molecular types. Restriction map for NTS VIII is not indicated.

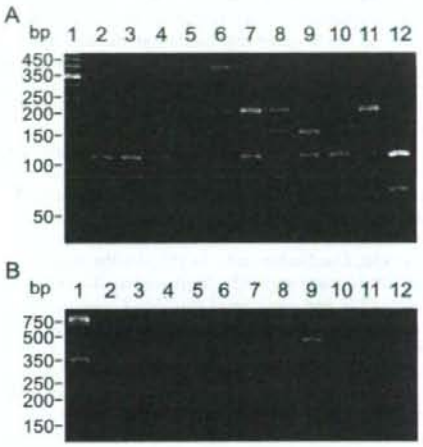

Fig. 2. Electrophoresis of PCR-RFLP products following restriction digestion with A: *Mva*I, and B: *Ava*I. Lane 1, Size marker; 2, KMU 3188; 3, KMU 3313; 4, KMU 4251; 5, KMU 4253; 6, KMU 4254; 7, KMU 4409; 8, KMU 4855; 9, KMU 4918; 10, KMU 6028; 11, KMU 6099; 12, CBS 129.35.

the NTS region, were digested with *Mva*I and *Ava*I (Toyobo) and then electrophoresed on 5% polyacrylamide gels. Based on the sequence data (8), restriction maps were generated for each molecular type (Figure 1).

Banding profiles of NTS from these 10 strains isolated from sporadic cases were classified into 7 molecular types, i.e., NTS IV (4 strains), and NTS I, II, V, and VI (1 strain each), and two types showing characteristic profiles not found in a previous study (8) (Figure 2, Table 1). NTS IV strains, which were never found among the strains isolated in the epidemic, were found in Niigata (6,12), Akita (7), and Chiba Prefectures (13). All of the cases were of tinea capitis in elderly patients aged between 62 - 74 years. None of the cases of NTS IV were related to foreign travel as a source of infection. NTS II (KMU 4409) was isolated from a boy with kerion who had lived in Singapore and had attended a nursery school where an epidemic of tinea corporis had occurred (14). Both cases of tinea capitis caused by NTS V (KMU 4855) and NTS VI (KMU 4253) were apparently related to travel or living abroad. KMU 4253 was isolated from a girl with tinea capitis from another immigrant family from Peru (15). KMU 4855 was isolated from a boy with tinea capitis who had stayed with his family in the USA, where he suffered from tinea (16). KMU 6099 was isolated from a 17-year-old female immigrant from Peru, who had suffered from scalp dandruff and was treated successfully with topical antimycotics at 8 years of age (17). When the amplicon from the strain was digested with *Mva*I, the banding profile was the same as that of NTS II. However, it showed a characteristic profile composed of 3 bands with *Ava*I digestion (Figure 2B, lane 11). We designated the new banding profile as NTS VII. Nucleotide sequence analysis indicated the amplicon from KMU 6099 to be 778 bp in length. The estimated sizes of the digested fragments based on the sequence of 731 bp of the region located at the center of the amplicon (DDBJ accession no. AB374179) were 208 bp (doublet), 115 bp, 90 bp (doublet), and 67 bp on digestion with *Mva*I, and 298 bp, 261 bp, and 219 bp on digestion with *Ava*I. The estimated banding profile was compatible with the results of electrophoresis (Figure 2B, lane 11). The nucleotide sequence of KMU 6099 was very similar to that of the NTS region of the *T. tonsurans* "variant V" ribosomal RNA gene reported by Gaedigk et al. (18) with 99.86% sequence identity (730/731 bp). Variant V was one of 5 molecular variants comprised of only 3 strains isolated from Columbus, Ohio, and Kansas City, Montana, among 92 strains

acquired from 6 US microbiology laboratories (18). The last strain, KMU 4254 was detected in an 8-year-old girl with kerion, but she had no history of foreign travel or family history of *T. tonsurans* infection (19). The strain was first identified as NTS II (8), but was later found to have a distinctive banding profile designated as NTS VIII, characterized by a band about 400 bp in length on digestion with *Mva*I digestion but no restriction site with *Ava*I digestion. The nucleotide sequence of the generated NTS fragment from KMU 4254 has yet to be determined.

Interestingly, only one strain from a case of tinea capitis in a 10-month-old girl showed the NTS I profile, which is the predominant molecular type in the epidemic in Japan. Of all 232 strains of *T. tonsurans* identified in a previous study (8), 199 were NTS I, and 98% of the 164 strains isolated from judo practitioners (160/164) were NTS I. The girl was later noted to be the daughter of a retired female judo practitioner, who had also been receiving treatment for the black dot type of tinea capitis (20). Therefore, this case was not truly sporadic but was related to sports, and was indeed an example of the secondary spread of an epidemic-related strain to the community.

Another point of interest is the historical aspect of infection with NTS IV strains. The NTS IV strains in the present study were isolated between 1989 and 2002 from rather elderly individuals. One old strain of *T. tonsurans* isolated in Japan was deposited in the Centraalbureau voor Schimmelcultures (CBS; Utrecht, The Netherlands) in 1935 by Professor M. Ota (21). The strain was originally deposited as *Bodinia spadix* (Kato 1925) (22), and was later changed to *T. spadix*, recorded as a synonym of *T. rubrum* in a standard textbook (23), but is now preserved in CBS as *T. tonsurans* CBS 129.35. The strain was found to be genotype I based on sequence analysis of the NTS region by Sugita et al. (21), and the strain was identified as NTS IV in our system (Figure 2). The first case of *T. tonsurans* infection in Japan was believed to be in a 50-year-old woman with kerion celsi in 1968 (4). However, our study of sporadic cases suggested that the history of *T. tonsurans* infection in Japan may date as far back as 1935. In addition, Urabe and Kawano (5) noted that the morphology, pathogenesis, and parasitism of the hair by *T. coccineum* (Kato 1925) (22), which is also described as a synonym of *T. rubrum* (23), showed considerable similarity to those of *T. tonsurans*. *T. coccineum* was found to be a rather common causative agent of tinea capitis among soldiers and students in Kyushu and Okinawa (22), Chiba, Hokuriku, including Kanazawa, Shizuoka, Osaka, Chugoku-Shikoku, and the northern part of Korea and Taiwan between 1925 and 1947 (24). Therefore, *T. tonsurans* may have been part of the domestic flora of Far East Asia in that era, including southwestern to central Japan, and its molecular type may be NTS VI. The complexity of the nomenclature of *B. spadix*, *T. coccineum*, and *T. tonsurans* should be simplified based on molecular markers, but no culture collection of *T. coccineum* is currently available.

In conclusion, this study suggested that NTS IV strains caused an endemic of sporadic or familial cases of *T. tonsurans* infection in Japan, and that later NTS II, V, VI, and VII were introduced independently from abroad. However, NTS I strains may become the most prevalent molecular type among strains isolated from sporadic cases, as NTS I is widespread among young people and because a secondary spread of the epidemic-related strain to the community has been confirmed.

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◆特集／皮膚真菌症診療ガイド
—これだけは知っておきたい皮膚真菌症の知識—

病原黒色真菌および類縁病原真菌の分離・同定について

佐野文子*　西村和子**

Key words：クロモミコーシス(chromomycosis)，黒癬(tinea nigra)，黒色酵母(black yeast)

Abstract　黒色真菌症原因菌は *Cladophialophora* 属，*Exophiala* 属，*Fonsecaea* 属，*Hortaea* 属および *Phialophora* 属菌種が主な病原性黒色真菌として挙げられ，同定は形態学的，生理生化学的および分子生物学的手法を組み合わせることが望ましい．

分離・同定方法

1．培　地

落屑痂皮や吸引物はそのまま，生検組織は数個に分割して培地上に置く．皮膚科で一般的に使用されている抗生物質を添加したサブロー寒天培地(Sabouraud's dextrose agar；SDA)やマイコセルアガーのスラントおよび平板，どちらでも構わないが，平板を用いたほうが後の純培養がしやすい．また，クロラムフェニコールを 100 mg/l で加えたポテト・デキストロース寒天平板培地(potato dextrose agar；PDA)でもよいが，培養初期の酵母様生育の判定に熟練を要する．分離された菌は環境からの混入か原因菌であるかを見極めることが重要である．

学会などの発表用には SDA と PDA での集落および PDA やコーンミール寒天培地で作製したスライドカルチャーによる顕微鏡所見が要求されることが多い．栄養源を 1/10 に減量した SDA や，市販の PDA 培地を 25～50%に減量し，寒天を所定濃度に補った培地も分生子形成がよい．

検査を外注する場合は室温送付を推奨する．患者検体および分離株ともに凍結は禁忌である．夏期以外は冷蔵もできれば避けたい．

2．培養温度・期間

25℃の孵卵器があれば使用するが，室温で差し支えない．複数の孵卵器が準備できるのであれば，25℃と 35℃を用いる．一部の菌種は 37℃で生育しないか，抑制される．培養期間は 4 週間以上，場合によっては 8 週間まで観察を続ける．通常の病原性酵母の分離のように 1 週間程度で観察をやめてはならない．菌種が推定された時点で最高生育温度を測定する．

3．同　定

形態学的な同定は，学会発表や論文投稿では教科書的に記載されている株との比較として重要である．

形態学的同定は集落の形態，光学顕微鏡による観察(菌糸の色，幅，隔壁，分生子の形態および着生状態)は必須である．場合によっては走査電子顕微鏡での形態学的観察が必要である．

また，生育最高温度，硝酸カリウム利用能など生理生化学的性状の記載も重要である．

しかし，患者分離株では形態学的観察が困難な場合も多いので，分子生物学的同定が必要である．また，最近では分生子形成を待たずに，集落が成育してきたところで，菌体の一部から DNA を抽出し，ribosomal RNA 遺伝子の internal transcri-

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図 1.
Fonsecaea pedrosoi
a：PDA，25℃，28 日間培養の集落
b：小型，無色，楕円形の分生子を生産しているフィアライド(矢印)によるフィアロ型分生子形成
c：クラドスポリウム型，リノクラジエラ型および中間型の分生子形成がみられる．

bed spacer(ITS)-1-5.8S-ITS2 領域および large subunit の D1/D2 領域の配列を決定し，BLAST サーチ(http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi)により迅速に菌種を推定する方法を平行して行うことが多い．病原性黒色真菌でのこの遺伝子領域の配列は GenBank のデータベースが充実しているので，データベース上での検索はたやすい．なお，発表には GenBank のアクセッション番号(http://www.ncbi.nlm.nih.gov/pubmed)および BLAST サーチの ID などが要求されることが多い．

主な病原性黒色真菌[1)~3)]

黒色真菌症の原因菌は約 30 属 60 種ほどの菌種がヒトや哺乳類，両生類，魚類，甲殻類などの動物に病原性を有する．

これらのうち重要な菌属は *Fonsecaea* 属の *F. compacta* と *F. pedrosoi*，*Cladophialophora* 属の *C. bantiana* と *C. carrionii*(後者は本邦で未報告．中国山東省，河北省，ベネズエラ，オーストラリアに分布)，*Exophiala* 属の *E. dermatitidis*，*E. jeanselmei*，*E. moniliforme*，*E. spinifera* および *E. xenobiotica*，*Hortaea* 属の *H. werneckii* および *Phialophora* 属の *P. verrucosa* が挙げられる．

ほかに本邦で分離されている菌種として *Alternaria alternata*，*Aureobasidium pullulans*，*Bipolaris spicifera*，*Exerohilum rostratum*，*Ochroconis gallopava*，*Phaeoacremonium parasiticum* (*Phialophora parasitica*)，*Ph. rubrigenum*，*Pleurostomophora* (*Phialophora*) *repens*，*Pl. richardsiae*，*Veronaea botryosa* などが挙げられる．

主な病原性黒色真菌の形態学的特徴

1．*Fonsecaea* 属

黒色真菌症原因菌として最重要菌種である．

a) *Fonsecaea pedrosoi*(図 1)

F. pedrosoi(国内で従来 *F. pedrosoi* とされた菌株は最新の分子分類では *F. monophora* に属するが，地域多型の一つとする意見もある．形態学的な差異はないので，ここでは *F. pedorosoi* として扱う)はクロモミコーシスの原因菌として最も多く，世界中で分離されている．

集落の生育は中等度である．SDA，27℃，14 日間培養で直径 2.5 cm 前後の黒色ないし黒緑色の集落を形成する．表面に灰色の短い気菌糸が密生することが多い．

顕微鏡所見は *Rhinocladiella* 様のシンポジオ型と *Cladosporium* 様の出芽型分生子形成がみられ，稀にフィアロ型分生子形成がみられる多形性真菌である．分生子は 1 細胞性，淡褐色～褐色，楕円形，長い倒卵形，円筒形，1.5～3.0×3～6 μm である．

b) *Fonsecaea compacta*(図 2)

分子系統では *F. pedrosoi* と同種とされるが，形態と生育速度は異なる．中南米，中国で少数，日本でも分離されている．生育は遅く，SDA，27℃，14 日

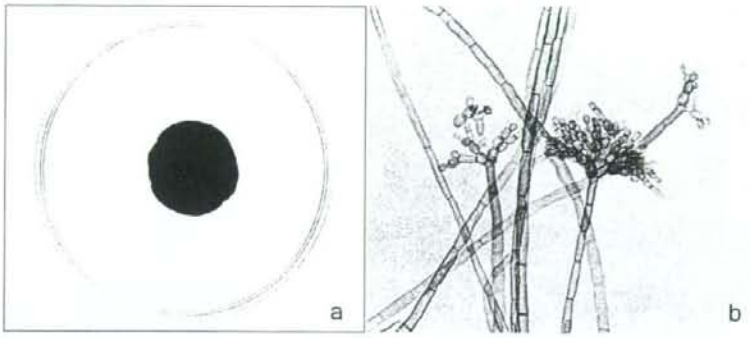

図 2.
Fonsecaea compacta
a：ポテト・デキストロース寒天，25℃，45日間培養
b：本種は主にクラドスポリウム型分生子形成を行い，分生子は亜球形，樽形，円筒形で互いに広い出芽痕で付着している．

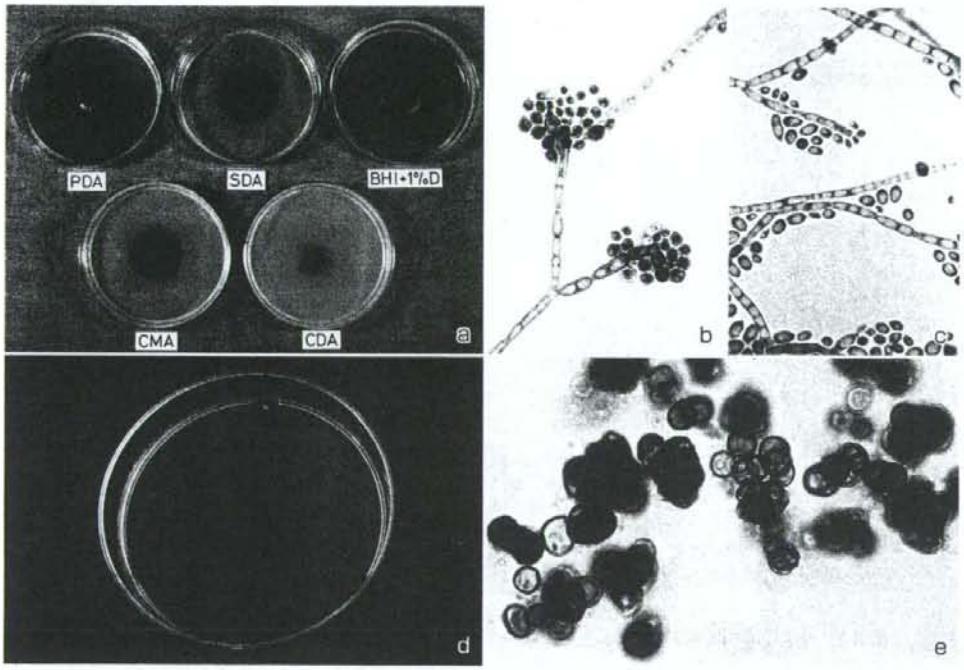

図 3. *Exophiala dermatitidis*
a：37℃，21日間培養後の集落．PDA：ポテト・デキストロース寒天培地，SDA：サブロー寒天培地，BHI+1% D1%ブドウ糖添加ブレイン・ハート・インフュージョン寒天培地，CMA：コーンミール寒天培地，CDA：ツァペックドック寒天培地
b，c：フラスコ形～円筒形の分生子形成細胞(アネライド)が球形，楕円形の分生子を産生
d：ポテト・デキストロース寒天培地，25℃，42日間培養の顆粒型集落
e：ブレイン・ハート・インフュージョン寒天培地，25℃，42日培養後に観察された黒褐色，厚壁の細胞顆粒型集落の顕微鏡所見

間培養で直径が1 cmに満たない黒色，表面に短菌糸が密生した集落を形成する．分生子形成は*F. pedrosoi*と同じく出芽型およびシンポジオ型が主で，稀にフィアロ型がみられる．分生子は*F. pedrosoi*より丸味を帯び，分生子どうしが密着する．

2．*Exophiala*属

培養条件により集落あるいはその一部が酵母様になるものがあり，黒色酵母(black yeast)と呼ばれる由縁である．本属の分生子形成はアネロ型のみである．なお，本属，特に*E. jeanselmei*と類似

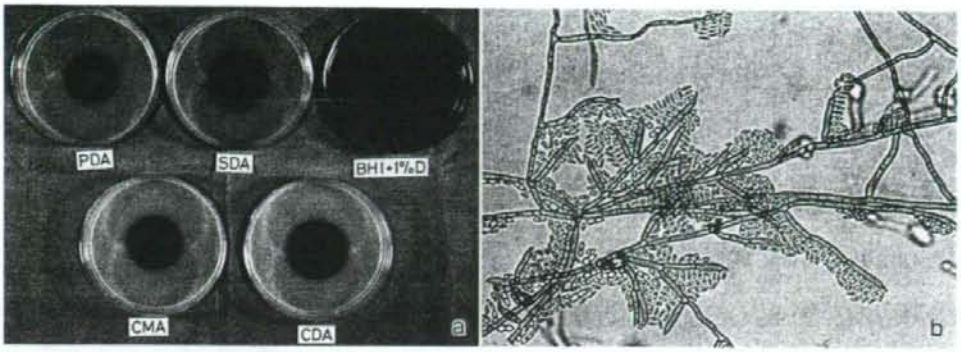

図 4. *Exophiala jeanselmei*

a：27℃，3週間培養の集落．*E. dermatitidis* と異なり，培養が進むとカビ状になる．37℃での生育は抑制されるか，生育しない．有機窒素を含まない合成培地 CDA でもよく生育する．

b：菌糸先端や側枝として生じた瓶状のアネライド，あるいは菌糸側壁の小突起から楕円形，長楕円形の分生子が生じる．

菌種の同定を最近の分類に従って行うためには ribosomal RNA 遺伝子の internal transcribed spacer(ITS)-1-5.8S-ITS2 領域および large subunit の D1/D2 領域の配列決定が必要である．

a）***Exophiala dermatitidis***(図 3)

本菌種による病変はクロモミコーシス様病変が多いが，一部は転移病巣として脳，肺，肝などの膿瘍，腫瘤様病変を合併し，極めて稀に心内膜炎もある．実験的に neurotropism が指摘されている．集落の生育は比較的遅い．37℃での生育は良好で，42℃でも生育可能である．病原性黒色真菌はほとんど硝酸カリウムを利用するが，本菌のみ利用できないため，ツァペック・ドックス寒天(CDA)上での生育は不良である．1%デキストロース加ブレイン・ハート・インフュージョン寒天(brain heart infusion agar)上で溶けたチョコレート状の集落を形成する．

光学顕微鏡的には菌糸形集落では円筒形，瓶形のアネライド(annellide)が菌糸先端あるいは側枝として生じる．アネライドは鎖状に連なることが多い．アネライドの先端にはレースの縁飾り状の環紋(通常 1～5 段)を持つ小突起がみられる．電子顕微鏡的には菌糸側壁からも環紋を持った小突起が生じて分生子を産生する．分生子は淡褐色～褐色，卵円形，楕円形，球形，1～3×1.5～4 μm である．

また，炭粉状，桑の実状集落(顆粒型集落)を形成する菌株では，褐色，厚壁の sclerotic body 様細胞の集塊あるいは連鎖がみられる．この細胞は縦横の隔壁で仕切られていることもある．

b）***Exophiala jeanselmei***(図 4)

抵抗性の減弱した患者に皮下膿瘍や表在性肉芽腫性病変を引き起こす．極めて稀に角膜真菌症の原因菌ともなる．諸外国では足菌腫の原因菌として知られている．

集落は始め酵母形，まもなく菌糸形生育を示す．気生菌糸がよく生育する菌株も多い．顕微鏡的には多少菌糸生育が良好な点とアネライドの先端がやや長い点を除けば *E. dermatitidis* とよく似ており，鑑別は難しい．有機窒素を含まない合成培地のツァペックドック寒天培地での生育がよいことが特徴である．最高生育温度 35～38℃．臨床分離株は 37～38℃が多いが，生育は抑制される．40℃では生育できない．

E. xenobiotica は 2006 年に *E. jeanselmei* 菌群から独立した菌種で，アネライド分生子形成部の着色がやや淡く，最高生育温度は 33～36℃で，*E. jeanselmei* よりやや低い[4]．個人的情報では，我が国でも症例が確認されている．

c）***Exophiala spinifera***(図 5)

皮膚，粘膜の肉芽腫性病変を引き起こす．集落は始め酵母形，まもなく菌糸形となる．アネライドは，長い瓶形，つぼ形など，アネライドを生じる菌糸は栄養菌糸より褐色調が強いことが特徴