

Changes in frequency of agents of tinea capitis in school children from Western China suggest slow migration rates in dermatophytes

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Tinea capitis is a common dermatophyte infection of the scalp of children in Western China, with the gray-patch form being the most prevalent. Twenty years ago, the most widespread etiologic agent was reported to be *Trichophyton violaceum*, which was later succeeded by *Microsporum ferrugineum* and *Trichophyton schoenleinii*. In the framework of our recent study, 97 isolates were collected from patients with clinically suspected tinea capitis. Identification was performed by conventional methods and by sequencing the ribosomal DNA internal transcribed spacer region. In the case of *T. violaceum* an additional microsatellite primer set (T1) was used. Five species (in order of frequency, *Trichophyton violaceum*, *T. schoenleinii*, *Microsporum ferrugineum*, zoophilic strains of *Arthroderma vanbreuseghemii*, and *Trichophyton tonsurans*) were identified. Results of molecular and phenotypic ID of the same strains showed good correspondence. Comparison with earlier data showed that dermatophytes species in former rural societies must have migrated extremely slowly. Preponderance of local transmission from domesticated animals was proven by the occurrence of zoophilic strains of *Arthroderma vanbreuseghemii*. Etiologic agents in the rural communities of Western China tend to be different from those of the other regions in the country, despite modern communication and traffic.

Keywords dermatophytes, China, epidemiology, taxonomy

Introduction

Tinea capitis, a dermatophyte infection of the scalp that primarily affects children, originates from different environmental sources. Over the past 40 years, epidemics of tinea capitis have occurred throughout the south of Xinjiang Province in Western China [1]. The most prevalent etiologic agent in cases of this infection during 1973–1990 was *Trichophyton violaceum* (47.95%), with *Microsporum ferrugineum* and *Trichophyton schoenleinii* being found at lower frequencies of

23.66% and 21.89%, respectively. *Trichophyton verrucosum*, *T. tonsurans* and *T. mentagrophytes* were uncommon with involvement in 0.15%, 6.26% and 0.09% cases, respectively. A significant decline in the total number of capital dermatophyte infections was observed in the late 1980s [1,2], probably, in part due to the introduction of griseofulvin. However, since 1995 a substantial increase in the number of cases has been observed, including notable changes in the spectrum of etiologic agents [3]. *Trichophyton schoenleinii* and *M. ferrugineum* are no longer detected and there is a higher incidence of cases caused by *T. violaceum* (50%), *T. tonsurans* (16%) and *T. verrucosum* (32%), respectively. The presence of the latter species is probably a result of the expanded sheep farming in these areas of China. It should be mentioned that the zoophilic

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species *T. verrucosum*, associated with cattle and sheep, traditionally consisted of two varieties, i.e., *T. verrucosum* var. *verrucosum* and *T. verrucosum* var. *autotrophicum*, the latter differing by vitamin dependence. Recent molecular and phenetic data, however, suggested that *T. verrucosum* var. *autotrophicum* is conspecific with *Arthroderma vanbreuseghemii* [4].

Previously published incidence data are based solely on the examination of the phenotypic features for the etiologic agents. This kind of diagnostics relies on the examination of colony and microscopic morphology, such as growth rate, colony pigmentation, size and shape of macro- and microconidia, and several physiological properties such as the production of urease, alkaline on bromocresol purple medium, assimilation of sorbitol and the requirement for certain vitamins or amino acids. However, due to pleomorphism, it is sometimes difficult to identify dermatophytes on these characteristics. Molecular methods have been shown to be superior in the identification of dermatophyte species. For example, sequencing of the rDNA internal transcribed spacer (ITS) region has successfully been applied [4]. For some species like members of the *T. violaceum*/*T. rubrum* complex, additional tests are available, e.g., using microsatellite markers as target [5].

To evaluate the current dermatophyte epidemiology in the south of Xinjiang Province of China, 189 patients with symptomatic tinea capitis were sampled in 2003. Some 97 strains were identified by comparing their physiological and morphological features with data obtained through the use of molecular methods.

Material and methods

Strains

Fifteen primary schools were visited in Uyghur communities in Western Xinjiang, China, approximately 50 km from Kashgar (Kashi) City (Fig. 1). Of 5204 Uyghur children examined, 189 were clinically diagnosed as having tinea capitis (Tables 1–3). Clinical hair specimens from this group of children, along with samples from their caps and scarves, as well as from the fur of 20 sheep were collected. All were microscopically

examined in KOH mounts and found to be positive for fungal elements. These specimens were also cultured on Sabouraud glucose agar (SGA) containing chloroamphenicol (50 mg/l) and cycloheximide (400 mg/l) and incubated at 27°C.

DNA extraction

Isolates were inoculated into 2 ml Sabouraud peptone-glucose broth and incubated at 25°C for 14–28 days. Mycelia were extracted using the FastPrep DNA Kit (MP Biomedicals). DNA was purified with GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences) as recommended by the manufacturer.

PCR and DNA sequencing

The ribosomal ITS region was amplified using universal primers ITS1 (5'-TCC GTA GGT GAA CCT GCG) and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC); PCR amplicons comprised about 1000 nucleotides. Recalcitrant DNA samples, mostly from suspected *T. violaceum* isolates, were amplified with primers flanking microsatellite regions (T1.forward 5'-GTA AGG ATG GCT AGT TAG GGG, T1.reverse 5'-TGG TCT GGC CTT GAC TGA CC) [5]. Reactions were performed in 35 µl volumes containing 10 mM Tris-HCl, 21 pmol of each primer, 50 µM concentrations of each dNTP, 1.5 U of Taq polymerase, and 1 µl of template DNA. Samples were amplified through 30 cycles as follows; initial denaturation for 10 min at 95°C, followed by denaturation for 30 sec at 95°C, annealing for 30 sec at 60°C, and extension for 45 sec at 72°C, with a final extension of 3 min at 72°C. The PCR product was purified with the Ultraclean Microbial DNA Isolation Kit. Sequencing of both strands (ITS and T1) were performed using BigDye Ready Reaction Mix combined with a Biosystem 3730 × DNA analyzer. Strains were evaluated by BLAST (GenBank sequence database, www.ncbi.nih.gov/genbank/index.html). Sequences of species specific reference strains (Fig. 2) were aligned with clinical isolates using CLUSTAL W and the substitution model was used to construct a phylogenetic tree using the software TREEFINDER [6].

Conventional identification

Colony and microscopic morphology were studied on SGA. Physiological tests included: (i) Production of urease in urea broth (Oxoid, Haarlem, The Netherlands) was assessed at intervals for up to 7 days at 27°C, (ii) growth on bromocresol purple-milk solids-glucose agar (BCP), read at several times during

Table 1 Isolation rates of strains from humans, animals and textiles.

Source	Hair	Textiles (caps and scarfs)	Sheep
Total samples	189	15	15
Culture positive	110	5	5
Molecular ID	90	3	4
Isolation rate	58.20%	33.33%	33.33%

Table 2 Initial morphological identification (ID), molecular identification (ITS, T1) and phenetic characters verified after sequencing of the 97 strains.

No. of strains	Source	ITS ID	T1 ID	Phenotypic specialties
5	Human	<i>A. vanbreuseghemii</i> (9)		Colonies velvety, folded
4	Sheep			
10	Human	<i>M. ferrugineum</i> (10)		Colonies heaped, wrinkled; conidia usually absent; hyphae broad with prominent cross walls (bamboo hyphae)
31	Human	<i>T. schoenleinii</i> (34)		Colonies growing rather slowly, often cracking and splitting the agar; antler-like hyphae and/or favic chandeliers present
3	Scarfs, caps			
40	Human	<i>T. violaceum</i> (18)	(22)	Colonies growing slowly, leathery, wrinkled, purple; sporulation absent
4	Human	<i>T. tonsurans</i> (4)		Colonies suede-like; microconidia of variable size

7 days of incubation at 27°C, and (iii) requirements for vitamins and amino acids to differentiate *T. verrucosum* from *A. vanbreuseghemii* (formerly *T. verrucosum* var. *autotrophicum*) were evaluated on *Trichophyton* agars No. 1, 3 and 4 (Difco, Detroit, MI, USA) at 27°C and read periodically up to a maximum of 4 weeks.

Results

A total of 97 of 120 strains that grew in culture were successfully sequenced but for the remaining isolates we were unable to generate PCR products. The 97 strains were identified as *Trichophyton violaceum*, *T. schoenleinii*, *M. ferrugineum*, zoophilic strains of *Arthroderma vanbreuseghemii* and *T. tonsurans* (Table 2, Fig. 2). Ten strains were found to be identical to the *M. ferrugineum* reference strains CBS 426.63 and CBS 497.48. Nine others that were morphologically identified as *T. verrucosum* had 100% ITS similarity to each other and were found to be identical to '*T. verrucosum* var. *autotrophicum*' reference strain CBS 100378 (AY 213692- *A. vanbreuseghemii*). ITS sequencing of this taxon revealed three fixed polymorphisms, viz. one substitution and one Indel and differed from *A. vanbreuseghemii* in three nucleotides. These results are consistent with data presented by Gräser *et al.* [4]. Four additional strains were identical to each other and to reference strains of *T. tonsurans* AY 213691, AY213690.

Only 18 strains of *T. violaceum* were identified successfully with ITS sequencing. In the remaining 22 strains suspected to be *T. violaceum* on the basis of their morphologic characteristics, ITS PCR was unsuccessful and they were identified using the specific microsatellite primer set T1 according to Ohst *et al.* [5]. A single genotype (GT, 10 repeats, with a substitution and an Indel of four nucleotides [GGCC]), matching type C of Ohst *et al.* [5] was found.

Results of physiological identification (BCP, urease, *Trichophyton* agars 1, 3 and 4) were in the main, the same as those obtained through molecular ID. Physiologic features were not significantly different from standard patterns found with *T. violaceum*, *T. schoenleinii*, *M. ferrugineum*, and *T. tonsurans*. All nine '*Trichophyton verrucosum*' strains proved to be vitamin independent, as described for *A. vanbreuseghemii* and *T. verrucosum* var. *autotrophicum*.

In summary, *Trichophyton violaceum* (40 strains, 41.24%) and *T. schoenleinii* (34 strains, 35.05%) were the preponderant etiologic agents of tinea capitis in Xinjiang school children investigated in this study. In addition, *M. ferrugineum* (10 strains, 10.31%), *A. vanbreuseghemii* (9 strains, 9.28%) and *T. tonsurans* (4 strains, 4.12%) were also associated with scalp infections.

All cases we investigated involved children in the age range from 6–12 years visiting elementary

Table 3 Clinical data and direct microscopy of the 90 strains isolated from humans and verified by molecular methods.

	<i>T. violaceum</i>	<i>T. schoenleinii</i>	<i>M. ferrugineum</i>	<i>A. vanbreuseghemii</i>	<i>T. tonsurans</i>
Black-dot:	4 (10%)	0	2 (20%)	1 (20%)	1 (25%)
Gray-patch:	36 (90%)	22 (71%)	8 (80%)	4 (80%)	3 (75%)
Favus:	0	9 (21%)	0	0	0
Male/female:	25/15	21/10	4/6	3/2	3/1
Direct microscopy:	endothrix, hyphae, arthrospores	endothrix, spores in chains, hyphae, arthrospores	ectothrix	ectothrix	endothrix

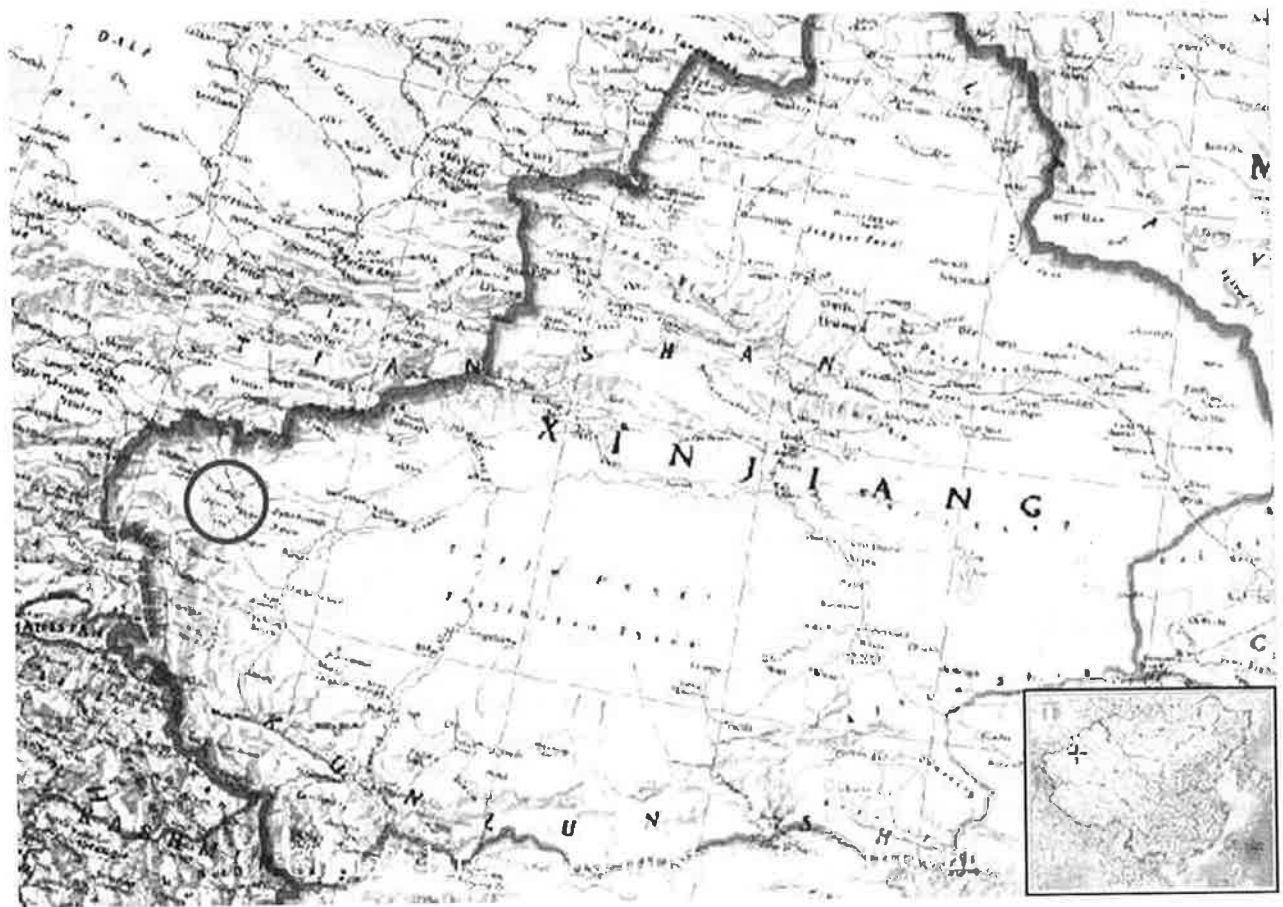


Fig. 1 Map of the study area. The circle indicates the location of the collection area of the samples within the Xinjiang province in Western China. The cross in the small picture (right lower edge) shows the location of the sample area within China.

schools, with symptomatic patients being predominantly male (m:f=1.5:1, Table 3). Gray-patch was the most common clinical form of the disease and *Trichophyton violaceum* the predominant agent, accounting for 40 cases of which 36 were gray-patch tinea capitis and the black-dot form in the 4 remaining individuals. *T. schoenleinii* accounted for 31 cases, of which 9 were favus and 22 gray-patch. Three strains isolated from patient's caps and scarves were identical to the species isolated from the respective patient's body sites. *M. ferrugineum* accounted for 10 cases, 8 of which were gray-patch and two black-dot forms of the disease. *A. vanbreuseghemii* was recovered from hair samples of 5 patients, 4 of which had gray-patch and one had black dot. Once a strain was isolated from the hand of a patient who also had tinea capitis. Of the four isolates of *T. tonsurans*, three were gray-patch and one black-dot disease.

Discussion

The Xinjiang province in Western China is relatively isolated as it is situated a great distance from the rest of the country. In addition, it is separated from the densely populated areas in the South by the Kunlun Mountains and the Taklimakan desert, as well as bordering on the North with Siberia. Tinea capitis is a relatively common fungal infection throughout the south of Xinjiang where its frequency has been monitored over the past 20 years [1]. During 1973–1990 the dermatophyte flora associated with this form of the disease was reported to consist of *T. violaceum* (47.95%), *M. ferrugineum* (23.66%), *T. schoenleinii* (21.89%), *T. tonsurans* (6.26%), *T. verrucosum* (0.15%) and *T. mentagrophytes* (0.09%). The identification of the etiologic agents was based on morphology and physiology. In the present study it was observed that conventional identification methods of agents of tinea capitis matched those obtained with molecular

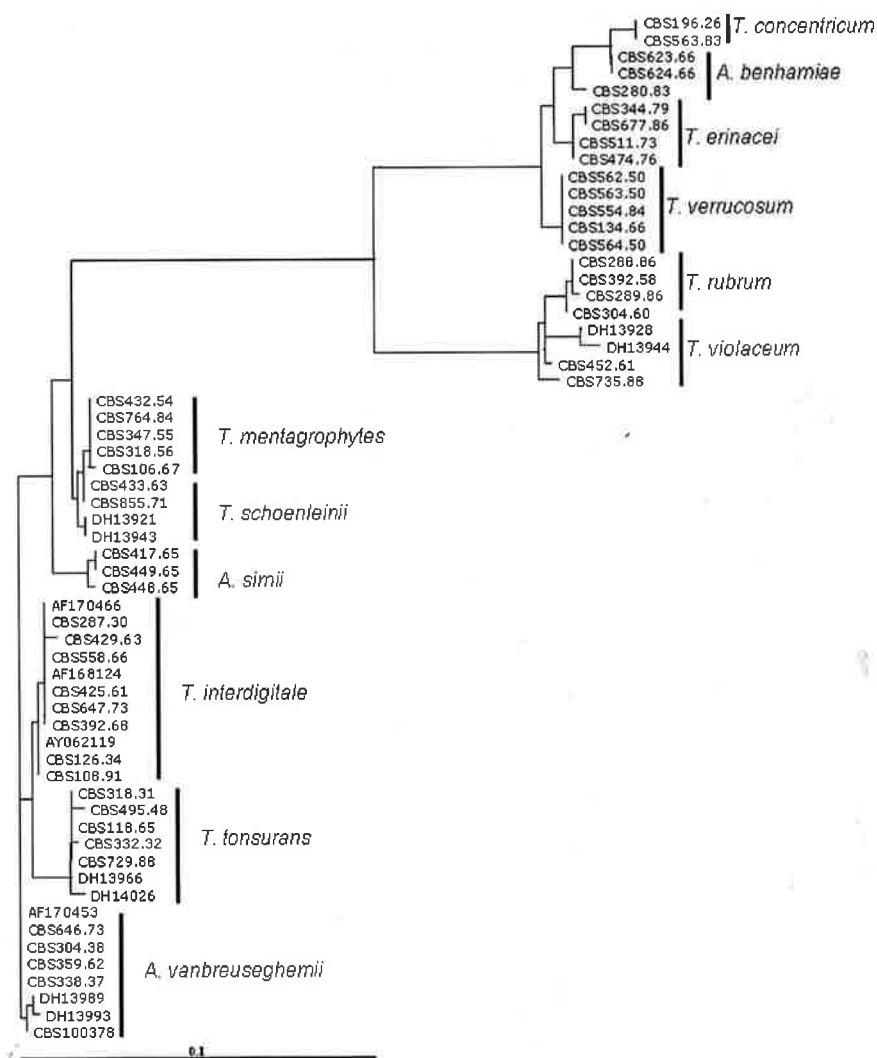


Fig. 2 Phylogenetic relationship of *Trichophyton* species under study using ITS sequences. The tree was generated using the GTRG substitution model and the Neighbor Joining method. Representative strains of each species from clinical sources are indicated by 'DH' numbers, CBS 338.37 was the type strain of *T. immersens*, CBS 359.62 of *T. balcanicum*, CBS 304.38 of *T. radicosum* and CBS 100378 of *T. verrucosum* var. *autotrophicum*. DH 139989 and DH 13993 are clinical strains that were isolated from sheep and a member of the family keeping the sheep, suggesting transmission.

diagnostics techniques (except for *T. verrucosum*). Consequently, the data collected over the period 1973–2003 with both procedures are largely comparable.

On the basis of ITS sequences, the results show that the species involved in tinea capitis has remained rather stable over the years, and that the province Xinjiang has maintained its distinctive flora over 20 years. This suggests that spread of dermatophytes still takes place at a slow pace. *T. violaceum* (40 strains, 41.24%) and *T. schoenleinii* (34 strains, 35.05%) are still among the preponderant species in the area investigated, although the latter has increased at the expense of *M. ferrugineum* (10 strains, 10.31%). This may in part be due to identification problems, given the close phenotypic similarity of *M. ferrugineum* and *T. schoenleinii* when grown on routine media. *A. vanbreuseghemii* (9 strains, 9.28%), which was identified as *T. verrucosum* in the

past, also has increased significantly and is associated with sheep rather than with cattle.

The etiologic agents of tinea capitis are quite characteristic of China. Wu *et al.* [2] collected data nationwide on the major etiologic agent of tinea capitis during the years 1986–1996. In 1986, *T. mentagrophytes* (which is known to be associated with three teleomorphic species, i.e., *A. vanbreuseghemii*, *A. simii* and *A. benhamiae* and *T. violaceum* were the predominant etiologic agents of tinea capitis in China, accounting for 52.3% and 17.4% of the reported cases, respectively. However, in 1996 *M. canis* replaced *T. mentagrophytes* as the primary etiologic agent of tinea capitis. The prevalent species involved in eastern parts of China [2] were *M. canis* (39.9%), *T. mentagrophytes* (22.5%), *T. violaceum* (10.1%), *T. rubrum* (9.4%), *C. albicans* (7.2%) and *T. tonsurans* (5.8%). *M. canis* was the most common organism isolated from hairs of the patients

with tinea capitis in the rest of China, while this species is absent from Xinjiang in the West. However, *A. vanbreuseghemii* strains previously identified as *T. verrucosum* var. *autotrophicum* or *T. mentagrophytes*, may occur throughout the country. It should be noted that the isolated '*T. mentagrophytes*' strains from the remaining areas of China may be anamorphs of *A. benhamiae* or close to *A. simii*.

The predominance of specific pathogens causing tinea capitis varies with geography, environment, climate, occupation and host lifestyle. Xinjiang Uyghur Autonomous region is located in northwestern China. It is bordered by the Kashmir region, Afghanistan, Tadjikistan, Kyrgyzstan, Kazakhstan, Russia, Mongolia and Tibet, and inside China by the Gansu and Qinghai provinces. Inhabited since early times by nomad tribes, it is an area of rugged mountains and desert basins. The Silk Road traversed the region and all of the southern area the region is composed of the Taklimakan desert. The climate there is dry, hot and seldom has any rainfall. Most of the residents are Uyghur people, who are Persian- or Turkish-speaking Muslims, and traditionally work with livestock farming. Probably for these reasons, the tinea capitis species spectrum of Xinjiang is significantly different from other regions in China and persists despite modern communication and traffic. Thus in the past, dermatophytes in rural societies must have migrated extremely slowly. Local transmission from animals was proven in the case of *A. vanbreuseghemii*.

Tinea capitis infections can be classified by their clinical appearance, such as gray-patch type, black-dot pattern, favus and inflammatory tinea capitis with kerion. In the province Xinjiang, gray-patch type is the most common form of tinea capitis. The clinical appearance of infections caused by *T. violaceum* is mainly non-inflammatory, like gray-patch, but typical black-dot pattern may also be found. Direct microscopic examination revealed endothrix hair invasion. Judging from marker T1 data, all strains collected were of type C (*T. violaceum*). Today *T. schoenleinii* is endemic to the Middle East, Asia and Africa [7]. It may cause tinea capitis but it has been speculated to have evolved from camels which live in desert-like environments [7]. Indeed Probst *et al.* [11] have shown that known camel-associated isolates of *T. mentagrophytes* (neotype), previously classified as *T. sarkisovii* and *T. langeronii*, may form favic chandeliers and have a very similar genetic make up when compared to *T. schoenleinii*, confirming the close relationship of both species. The preponderance of *T. schoenleinii* and the presence of camels in the arid climate of Xinjiang match with this hypothesis.

Clinical pictures frequently deviated from expected appearance as for example among 31 cases caused by *T. schoenleinii*, only nine of which were unambiguously identified as favus. Direct microscopic examination revealed endothrix hair invasion, but only hyphae and not spores were seen inside hair. This item needs further study. *Trichophyton schoenleinii* was cultured from infected hair, caps and scarves of symptomatic patients, suggesting that human-to-human contact was the key route of transmission.

A. vanbreuseghemii continues to increase in incidence in the area investigated in this study. Like *T. verrucosum*, *A. vanbreuseghemii* strains are regarded to be zoophilic, but differ from the former by being primarily associated with sheep rather than with cattle [8]. Human strains are supposed to have originated by transmission of strains from animals. The genetic make-up of human-associated strains should therefore be identical to that of the animal strains from which they were derived. The clinical appearance of infections caused by *A. vanbreuseghemii* strains was non-inflammatory in our cases. This is in sharp contrast to the highly inflammatory infections caused by *T. verrucosum*, usually resulting from transmission from cattle [9]. The reason may be that the anthropophilic species *T. interdigitale* and *T. tonsurans* are phylogenetically closely related. Direct microscopic examination revealed ectothrix hair invasion. Cultures were white, downy, poorly sporulating, but producing abundant chlamydospores. In addition, the fungus could grow well on media without vitamins, e.g., *Trichophyton*-agar No. 1. These are features consistent with the original descriptions of *T. verrucosum* var. *autotrophicum*, *T. balcaneum*, *T. immergens*, and *T. radicosum* [10]. Thus all of these species are considered as synonyms and their ITS sequences consistently clustered with *A. vanbreuseghemii* (Fig. 2). Consequently, strains ascribed to the four taxa were either isolated from sheep (*T. verrucosum* var. *autotrophicum*), or from geographical origins linked by the Silk Road, like Xinjiang (e.g., the Balkan = *T. balcaneum*, *T. immergens*). We also isolated from symptomatic family members four strains of *A. vanbreuseghemii* with genotypes identical to those of sheep, thus strongly suggesting transmission of this infection from domestic animals. Whether or not *T. mentagrophytes* strains isolated outside Xinjiang are also close to *A. vanbreuseghemii* remains to be investigated.

Microsporium ferrugineum ranks third after *T. schoenleinii* in Xinjiang whereas it is rare in other areas of China. Clinical appearance was non-inflammatory and direct microscopic examination revealed ectothrix hair invasion. Other agents of tinea capitis include

Trichophyton tonsurans, observed in four cases in our investigation. They were all non-inflammatory.

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References

- 1 Tai SL, Tian SR, Dong YR. Survey report of pathogens of tinea capitis in Xinjiang, China. *J Derm Venereol* 1992; **6**: 218.
- 2 Wu SX, Liao WQ, Guo NR, *et al.* Study on pathogenic fungi in China: a dynamic epidemiological survey between 1986 and 1996. *J Clin Dermatol* 1999; **28**: 1–5.
- 3 Deng SW, Li RY, Hui Y, Wang DL. Identification of strains isolated from tinea capitis in Xinjiang. *Chin J Dermatol* 2005; **38**: 173–174.
- 4 Gräser Y, Kuijpers AFA, Presber W, De Hoog GS. Molecular taxonomy of *Trichophyton mentagrophytes* and *T. tonsurans*. *Med Mycol* 1999; **37**: 315–330.
- 5 Ohst T, De Hoog GS, Presber W, Stavrakieva V, Gräser Y. Origins of microsatellite diversity in the *Trichophyton rubrum*-*T. violaceum* clade (dermatophytes). *J Clin Microbiol* 2004; **42**: 4444–4448.
- 6 Jobb G, von Haeseler A, Strimmer K. TREEFINDER: a powerful graphical analysis environment for molecular phylogenetics. *BMC Evol Biol* 2004; **4**: 18–26.
- 7 Verenkar MP, Pinto MJ, Rodrigues SJ, Singh I, Rege VL. Tinea capitis due to *Trichophyton schoenleinii*. *Indian J Pathol Microbiol* 1991; **34**: 299–301.
- 8 Scott DB. A new variety of *Trichophyton verrucosum*. *Trans Br Mycol Soc* 1976; **67**: 342–344.
- 9 Kane J, Summerbell RC, Sigler L, Krajdien S, Land G. *Laboratory Handbook of Dermatophytes*. Belmont, CA: Star Publishing, 344 pp.
- 10 Gräser Y, Kuijpers AFA, Presber W, De Hoog GS. Molecular taxonomy of the *Trichophyton rubrum* complex. *J Clin Microbiol* 2000; **38**: 3329–3336.
- 11 Probst S, De Hoog GS, Gräser Y. Development of DNA markers to explore host shifts in dermatophytes. *Stud Mycol* 2003; **47**: 57–74.

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