We present a familial infection caused by *Arthroderma vanbreuseghemii*. The proband is a 4-year-old boy, who had played with rabbits at his rabbit-farm neighbor. He complained of pruritus and pain in his scalp, which displayed redness, alopecia and painful cysts and eventually discharged pus and scabbed. Several erythema on his face and abdomen were also presented. He was diagnosed as having impetigo but antibacterial agents were not effective and his clinical condition did not improve. Several days later, his parents also developed facial erythema and scaling. The development of a kerion in the boy and tinea corporis in his parents were diagnosed based on the positive KOH examination. Morphologic and biochemical characteristics confirmed that their infections were caused by the zoophilic *Trichophyton mentagrophytes*, while sequencing of the internal transcribed spacer (ITS) 1/4 polymerase chain reaction products, amplified from primary culture isolates, established its *Arthroderma vanbreuseghemii* lineage. Random amplified polymorphic DNA (RAPD) analysis indicated these isolates might be the same strain and that infectio cruciata occurred in this family. Semi-quantitative analysis of these strains indicated multiple and main enzymatic activities of alkaline phosphatase, β-glucosaccharase. The boy was cured through treatment with itraconazole 100 mg/day orally in combination with topical washes with 2% ketoconazole shampoo, and his parents were successfully treated by topical application of terbinafine cream.

**Keywords** *Arthroderma vanbreuseghemii*, kerion, itraconazole, ketoconazole, terbinafine

**Introduction**

Kerion is an inflammatory or suppurative type of tinea capitis caused by zoophilic (*Trichophyton mentagrophytes* and *Microsporum canis*), as well as geophilic (*M. gypseum*) and anthropophilic (*T. tonsurans* and *T. rubrum*) dermatophytes. The major dermatophyte, *T. mentagrophytes*, is a complex species consisting of more than three teleomorphs, i.e., *Arthroderma benhamiae, A. simii* and *A. vanbreuseghemii* [1,2], and a number of anamorphs, i.e., the asexual forms [3]. In this study we described an intra-familial transmission of *A. vanbreuseghemii* in China, in which we sequenced the isolates recovered from the patients and found that they might have come from the same source. The combination of the oral use of antifungals, along with washing and topical application of the drugs resulted in the cure of all three patients.

**Case report**

The proband, a 4-year-old boy (19 kg body weight), living in Jintang county of Sichuan province, was referred to our clinic with a 10-day history of erythema,
scaling, pustule and inflammatory subcutaneous nodule lesions on his scalp and erythema on his face and trunk. He complained of itching, pain and fever and shortly afterwards suppuration and crustation developed on the scalp due to scratching. Upon hospitalization, physical examination of the boy revealed: (1) multiple erythema, alopecia, broken hair, scaling, 1- or 2-cm purulent lesion; (2) sub-crustation and subcutaneous cysts on the parietal region and occiput (Fig. 1a); (3) mild erythema and scale lesions on the face, right scapular region and left hypogastrium. His temperature was 40.1°C, pulse rate was 110 beats/min, and respiratory rate was 21 breaths/min. Soon after the boy’s admission, his father and mother were seen as the result of a 5 day history of erythema with scales on their faces (Fig. 1c). One week prior to the current observations, the boy was diagnosed with impetigo by a nearby dermatologist and treated by debridging, systemic and topical antibacterial agents (specific information unavailable), but the lesions became severe and he had further fervescence accompanied with chills and ague. Before his illness, in his neighbor’s rabbit-farm, the boy had frequently played with rabbits. Some of the animals had the signs of depilation but we couldn’t confirm that these were fungal in origin due to the farmer’s refusal to allow us to examine the rabbits. His parents didn’t touch any animals. The boy was otherwise healthy without any immunosuppressive disorder and had no significant medical history.

Laboratory examination

Complete blood cell count showed a leukocyte count of 22.87 × 10⁹/l (normal 4–10 × 10⁹/l) (86% segmented neutrophils (50–70%), 10% lymphocytes (20–40%), and 4% stab nuclear neutrophil (1–5%)), hemoglobin level of 118 g/l (120–160 g/l), and platelet count of 363 × 10⁹/1 (100–300 × 10⁹/l). Biochemical testing showed renal function, liver enzymes, and electrolyte levels were all within normal limits. Culture of blood and secretion from scale lesions was negative for bacteria.

Mycological examination

The direct mycological examination of 10% KOH mounts of the boy’s hair showed extremely high numbers of ectothrix spores (Fig. 1b). Additional 10% KOH preparations of skin scrapings from the boy and his parents’ face lesions also revealed hyaline hyphae. Cultures inoculated with samples from the boy’s scalp and his parents face lesions were incubated

Fig. 1 Lesions of the boy’s occiput (a) and microscopic examination of the boy’s hair revealed numerous ectothrix spores (b, ×400). Facial erythema with scales on his father (c).
at 28°C on Sabouraud glucose agar (SDA, Oxoid Ltd, Hampshire, UK) containing chloramphenicol and cycloheximide anide all yielded thin, floccose and white colonies with deep yellow margins and orange reverse pigmentation. Microscopic examination of lactophenol cotton blue stain preparations from slide cultures revealed fertile and septate hypha, spherical to tear-shaped microconidia and clavillose, thin-walled and laevis macroconidia without spiral hyphae (Fig. 2). Urease activity on urea agar slant (Oxoid Ltd, Hampshire, UK) incubated at 25°C was assessed every day for 7 days, and in vitro hair perforation tests were performed using human infant hairs (Fig. 3). The hairs were cut into approximately 2 cm lengths and sterilized by autoclaving. Three milliliters of a culture medium containing 0.1% peptone and 0.2% glucose was decanted into 3 cm plastic Petri dish and inoculated with approximately 10 hairs and a loop of fungal mass. The plates were incubated at 25°C for 3 weeks. After this period of time, several hairs were removed and observed under a light microscope. Three strains isolated from family members were identified as *T. mentagrophytes* on the base of these morphologic and biochemical features.

The analysis of DNA sequences and RAPD

Three isolates, one recovered from the boy’s scalp and one from each of his parents’ faces, were cultured on SDA at 28°C for one week, and the primary cultures were used for the analysis of DNA sequences. Rapid preparation of DNA from moulds was performed by the method described by Makimura [4]. The general fungal primer pair ITS1 (5’-TCCGTAGGTAACCTGCGG) and ITS4 (5’-TCCTCCGCTATTGATA TGC) (Shanghai Invitrogen Biotech Co. Ltd, China) was used for the amplification of a single product encompassing portions of the small and large nuclear rDNA sequences and the entire intervening ITS1 region, the 5.8S rDNA gene, and the ITS2 region [5]. Each 50 μl reaction mixture contained 25 μl PCR pfuMix (dATP, dCTP, dGTP, dTTP and pfuDNA Taq polymerase; Beijing Tiangen Biotech Co. Ltd, China), 2 μl of each primer, 17 μl ddH2O and 4μl DNA sample. The PCR mixture was denaturalized at 94°C for 2 min, 40 cycles of denaturation at 94°C for 1 min, annealing at 34°C for 1 min, extension at 72°C for 2 min, and final extension at 72°C for 10 min. Three amplification products were detected as a single band by 2% agarose gel electrophoresis and UV irradiation and sent to Invitrogen Life Technologies for DNA purification and bidirectional sequencing. The sequences were aligned (BIOEDIT, http://www.mbio.ncsu.edu/) and deposited in the GenBank with the accession number EU683894 (isolated from son, 666bp), EU683893 (isolated from father, 663bp) and EU683892 (isolated from mother, 686bp). These DNA sequences of nuclear ribosomal ITS region of the isolated fungi were all in accordance with *A. vanbreuseghemii* RV 27961 (DDBJ/EMBL/GenBank accession No. AF170453.1) with the homology of 99% and 100% by using the Blast 2 Sequences Tool (http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi). Judging from these sequence data, our isolates were confirmed to be *A. vanbreuseghemii* and could be the same strain. To investigate the latter, RAPD was performed with the same DNA extraction and a known isolate of *A. vanbreuseghemii* was used as a control. This isolate had been isolated from another patient and had been identified based on its DNA sequence of the ITS region. Since we could not find a paper in the literature describing RAPD analysis of *A. vanbreuseghemii* strains, there were no primers which had been proved to have the discriminatory

![Fig. 2](image_url) Microscopic examination of slide-culture from the boy (a), his father (b) and his mother (c). Spiral hypha was not observed (stained by lactophenol cotton blue, ×400).
power to be selected for our studies. We used 20 10-mer primers selected randomly from OPI and OPK series primers (Operon Inc., Alameda, CA, USA), of which three oligonucleotide primers (OPI-3, OPI-17 and OPK-20) could produce polymorphic and distinct bands. The RAPD assay was performed in a total reaction volume of 50 µl, containing 25 µl PCR pfuMix, 2 µl of each primer, 18 µl ddH2O and 3 µl DNA sample. The reaction was performed according to the following protocol: one cycle of denaturation at 94°C for 40 sec, 45 cycles of denaturation at 94°C for 20 sec, annealing at 35°C for 1 min, extension at 72°C for 10 min, and final extension at 72°C for 10 min. The amplification products were visualized by UV light after separation by electrophoresis on 1.5% agarose gel and staining with ethidium bromide. The four *A. vanbreuseghemii* isolates showed distinct and similar band patterns. The three isolates from our patients couldn’t differentiate from each other but could distinguish from the control *A. vanbreuseghemii*, which suggest that our isolates might have come from the same source (Fig. 4).

**Secreted enzymatic activities analysis**

The enzymatic activities of the isolated strains were determined with the semi-quantitative Api-Zym system.
buffer and enzyme substrate) on the strips provided was added to each of the individual cupules (containing sodium chloride solution. Then, 65 μl of the suspension was added to each of the individual cupules (containing buffer and enzyme substrate) on the strips provided and incubated at 37°C for 4 h. The pre-supplied color reagent (Fast Blue BB, lauryl sulphate) was added to determine enzymatic activity. β-glycosidase (Sigma) was performed as quality control and autoclaved microorganisms were used as negative controls. The color alterations were scored relative to the chart provided by the manufacturer. Only those readings significantly different from the negative control were scored as positive. The enzymatic activity was judged, according to the degree of color change (Table 1). The three strains showed the same pattern of enzyme type and activity, and these results also indirectly indicated they might come from the same source.

Treatment and follow up

In three family members, the kerion in the boy and tinea corporis in his parents were diagnosed separately. The boy was started on oral therapy with 100 mg/day itraconazole capsule (Sporanox, XIAN-JANSSEN Pharmaceutical Ltd), associated with topical washes with 2% ketoconazole shampoo (Triatop, XIAN-JANSSEN Pharmaceutical Ltd), and povidone iodine solution. Meanwhile, due to suppressing inflammation, intravenous drip with ceftriaxone sodium 500 mg/day and dexamethasone 7.5 mg/day were also administered for 6 days. After starting treatment, fervescence, pustules and most of cysts disappeared at the 3rd, 7th and 16th day, respectively. A cure was also achieved after 10 days for the lesions on the face, right scapular region and left hypogastrium. Complete blood cell count also became normal 6 days later. Antimycotic treatment continued and the kerion healed 3 months later, leaving residual scarring alopecia (Fig. 5). During the course of treatment, no side effect such as functional lesion of liver and kidney was found. The parents were healed 1 week after the onset of topical terbinafine cream treatment. For these infected members, no recurrence has been observed to date.

Discussion

Dermatophytoses in rabbit farming constitute an important reservoir for human infection with recurring disease reported in workers. The most frequent agents of such infection are members of the T. mentagrophytes complex, followed by M. canis and M. gypseum [6,7]. Infections in humans by Trichophyton spp. have recently emerged throughout the world [6–10]. In China, A. vanbreuseghemii infections have increased significantly and are associated with animals [8,9]. In the present cases, kerion and tinea corporis in the boy were found to be caused by a member of the T. mentagrophytes complex, which through DNA sequencing of the nuclear ribosomal ITS region was discovered to be in accordance with A. vanbreuseghemii. We speculate that the boy was infected by contact with his neighbor’s rabbits. These data of 100% homology among the isolates recovered from the child and parents, contact history, results of RAPD, and the same enzymatic type and activity all indicate that these strains may share the same source. Rabbits were probably the primary source of infection, which was then spread further by human-to-human contact.

A. vanbreuseghemii might have been brought over and spread by transportation and commercialization of animals and has since become a widespread zoophilic dermatophyte species [8]. Furthermore, A. vanbreuseghemii infections constitute serious problems for humans regardless of contact with animals, as evidenced by an outbreak of indirect transmissions of the fungal species among schoolchildren who had no history of contact with exotic and/or any other

Table 1  The results of assayed enzymatic activities.

<table>
<thead>
<tr>
<th>No.</th>
<th>Enzyme assayed</th>
<th>Result</th>
<th>No.</th>
<th>Enzyme assayed</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>-</td>
<td>11</td>
<td>Acid phosphatase</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Alkaline phosphatase</td>
<td>+ + + +</td>
<td>12</td>
<td>Naphthol-AS-Bi-phosphohydrolase</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Esterase (C4)</td>
<td>+ +</td>
<td>13</td>
<td>α-galactosidase</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Esterase lipase (C8)</td>
<td>+ +</td>
<td>14</td>
<td>β-galactosidase</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Lipase (C14)</td>
<td>-</td>
<td>15</td>
<td>β-glucuronidase</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Leucine arylamidase</td>
<td>+ + +</td>
<td>16</td>
<td>α-glucosaccharase</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Valine arylamidase</td>
<td>-</td>
<td>17</td>
<td>β-glucosaccharase</td>
<td>+ + + +</td>
</tr>
<tr>
<td>8</td>
<td>Cystine arylamidase</td>
<td>+ +</td>
<td>18</td>
<td>N-acetyl-glucosaminidase</td>
<td>+ +</td>
</tr>
<tr>
<td>9</td>
<td>Trypsin</td>
<td>-</td>
<td>19</td>
<td>α-mannosidase</td>
<td>+ + + +</td>
</tr>
<tr>
<td>10</td>
<td>α-chymotrypsin</td>
<td>-</td>
<td>20</td>
<td>β-fucosidase</td>
<td>-</td>
</tr>
</tbody>
</table>
A. vanbreuseghemii strains would seem to have a strong ability to be transmitted and survive among humans. Identifying pets, such as rabbits, guinea pigs, dogs and cats [9,10], as the sources of infections for people can help in the prevention of reoccurrence or establishment of new infections, especially in children, by adequately treating affected pets and their environments. It is also important to recognize the direct and indirect contact as an element in dermatophyte infections to successfully control these diseases in farm animal and human populations.

Semi-quantitative analysis indicated multiple enzymatic activities involving alkaline phosphatase, β-glucosaccharase, leucine arylamidase, α-mannosidase, esterase (C4), N-acetyl-glucosaminidase, esterase lipase (C8), cystine arylamidase, α-glucosaccharase, acid phosphatase, naphthol-AS-BI-phosphohydrolase. These main secreted enzymes are similar to those present in some reports [11,12]. Extracellular phospholipases activities have been proposed as virulence factors in dermatophytes, but there is limited information on these enzymes relative to A. vanbreuseghemii. Multiple enzymatic activities may be responsible for pathogenesis of A. vanbreuseghemii, i.e., their activity on different kinds of substrates for the nutrition of the organism growth may result in the damage of host tissue such as hair and skin.

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