Clonal outbreak of *Trichophyton tonsurans* tinea capitis gladiatorum among wrestlers in Adana, Turkey

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Tinea capitis gladiatorum and tinea corporis gladiatorum caused by the anthropophilic dermatophyte *Trichophyton tonsurans* are well-known clinical entities in individuals involved in combat sports e.g., wrestlers and judo practitioners. We present an outbreak of *Trichophyton tonsurans* tinea capitis gladiatorum among wrestlers at a boarding school in Adana, Turkey. Fourteen of the 29 wrestlers examined (48.3%) harbored the pathogen, including eight asymptomatic scalp carriers, five with tinea capitis superficiais, and one asymptomatic trunk carrier. Dermatophytes were isolated from samples of the neck (1), nape (1), trunk (3) and inguinal area (2) in four of the five tinea capitis cases. A total of five inanimate objects, i.e., two wrestling mats, two pillowcases, and one sheet were also found to be positive for *T. tonsurans*. Mixed-marker strain typing examining 24 sequence variations in 12 gene loci confirmed that the outbreak was caused by a single strain of *T. tonsurans*.

**Keywords**  asymptomatic carrier, tinea capitis gladiatorum, tinea corporis gladiatorum, *Trichophyton tonsurans*, outbreak

**Introduction**

*Trichophyton tonsurans* Malmsten, a causative agent of tinea capitis, was described as a predominant pathogen in Mexico as early as the 1940s [1] and gained in importance in the United States and Canada after the 1970s [2–4]. Moreover, cases involving the fungus are emerging in most parts of the world, e.g., United Kingdom [5,6], Spain [7], Japan [8,9], and Haiti [10]. It is also the major dermatophyte species related with asymptomatic scalp carriage [11]. In addition, Abdel-Rahman et al. [12] noted that the undefined factors that permit *T. tonsurans* to avoid clearance and to remain on the human host in a subclinical state contributed to the ability of the organism to effectively persist and thrive in the host population. Outbreaks of *T. tonsurans* are well-known in the literature, and widely discussed especially in combat sports, e.g., wrestlers [13–18] and judo practitioners [8,9]. It is also an agent of nosocomial infections [19,20].

In this investigation, we report an outbreak of scalp infections caused by *T. tonsurans* among wrestlers in Adana, Turkey. Molecular strain typing was employed to examine the genetic relatedness of the recovered isolates and their relationship to previously typed isolates from across the globe. Taxonomic reports in the literature are also updated with respect to modern concepts in order to prevent errors and anachronisms [21,22].

**Material and methods**

**Data collection**

This study was conducted from December 2008 to May 2009. Two athletes of a 29 member team at the İsmet Atlı Wrestling Boarding School presented with scalp lesions, e.g., broken hairs and alopecia, on the same day to the Department of Family Medicine clinic in Adana, Turkey. These wrestlers declared that some of their team members
had similar complaints at which point we examined the rest of the team to evaluate the presence of infection or carriage. Wrestlers were classified as cases, carriers or uninfected based on the combination of clinical and mycological findings. Wrestlers from whom dermatophytes were recovered but lacked clinical symptoms were considered to be asymptomatic carriers. The clinical diagnosis, mycological results, and detailed history of each wrestler were recorded.

Sample collection
Scalp samples were taken from all wrestlers, irrespective of the clinical symptoms, by vigorously brushing each side of the scalp four times with a plastic hairbrush. The hairbrush consisted of 167 plastic prongs, was circular in shape, and of a size that it would fit in a Petri dish. This method was also carried out for obtaining trunk samples irrespective of whether clinical lesions were present. For sampling of the inguinal area and toewebs, the cotton swab procedure was carried out after the swabs had been dipped in sterile saline. Samples were also collected from inanimate objects, e.g., the pillowcases ($n=29$) and sheets ($n=29$) of the wrestlers, as well as from the different parts of the wrestling mats ($n=26$). The study was reviewed and approved by Faculty of Medicine’s Ethics Committee of Cukurova University. Informed consent was received from the legal guardians of the wrestlers.

Fungal culture
Scalp and trunk specimens were dislodged when the brushes were inoculated onto the agar surface. Sabouraud glucose agar (SGA; Acumedia, Baltimore, MD, USA) plates containing 100 μg/ml cycloheximide (Sigma, Steinheim, Germany), 100 μg/ml chloramphenicol (Fluka, China), and 50 μg/ml gentamicin (Sigma) were used as a study medium. Each hairbrush were stabbed onto the medium, creating 167 inoculation points corresponding to the 167 prongs of the hairbrush. The cotton swab was inoculated onto the study medium by rotating the swab head while streaking the surface of the medium. All plates were transferred to the Mycology Laboratory at the Faculty of Medicine, University of Cukurova. The cultures were incubated at 25°C on the bench and were examined after 7, 14, and 21 days for evidence of growth [23].

Spore load
Colonies were counted on each plate for the hairbrush method, and a total colony count (equivalent to the number of spores retrieved) was obtained for each participant. A spore load system was assigned as follows: light for 1–5 colonies, moderate for 6–10 colonies, and heavy for >10 colonies [11,23,24] per plate.

Treatment
Patients with tinea capitis superficialis (TCS) and asymptomatic carriers were treated for 4 weeks with oral terbinafine according to body weight (20–40 kg, 125 mg daily; ≥ 40 kg, 250 mg daily). In addition, lesions of the scalp and glabrous skin were treated with 1% terbinafine cream twice daily for 4 weeks. Clinical and mycological follow-up was performed four and eight weeks after the initiation of treatment. For decontamination of the fomites, the wrestling mats had been cleaned with diluted sodium hypochlorite solution before every training practice, and pillowcases and sheets were washed at 70°C for 30 min twice a week. Inanimate objects were also re-evaluated for the presence of dermatophyte fungi at four months.

Molecular study
DNA isolation was performed according the protocol described by Turin et al. [25]. All isolates underwent molecular characterization using 24 sequence variations in the following 12 gene loci: the non-transcribed spacer (NTS) of the ribosomal RNA (rRNA) locus, alkalineprotease-1 (ALP1), metalloprotease-5 (MEP5), carboxypeptidases Y and M14 (CarbY, CarbM14), leucine aminopeptidases 1 and 2 (LAP1, LAP2), dipeptidylpeptidase IV (DPP4), subtilisine-like proteases 2, 3 and 5 (SUB2, SUB3, SUB5) and ceramidase. Details on the amplification conditions and enzymes used to determine these sequence variations have been previously described in detail [20,26–29].

Results
The mean age of the wrestling team was $15.4 \pm 1.4$ years. Out of the 26 T. tonsurans strains recovered from this outbreak, 21 originated from a human body site and five from inanimate objects (2 wrestling mats, 2 pillowcases, and 1 bed sheet). Molecular analysis revealed that all 26 isolates recovered in this investigation shared an identical genetic profile. The sequence variations observed at each locus are detailed in Table 1.

Dermatophytes were isolated from 48.3% (14/29) of the children, of whom 9 were carriers (31.1%) and 5 represented TCS cases (17.2%). The remaining 15 wrestlers (51.7%) were found to be neither infected nor carriers. Asymptomatic scalp carriage was found in eight wrestlers and trunk carriage was observed in an additional wrestler. One to three separate concurrent infected sites were noted in 4 of the 5 TCS patients. Most clinical sites involved the chest (3) followed by inguinal area (2), neck (1), and nape of the neck (1).

The number of colonies resulting from the 167 prong hairbrush stabbed into the culture medium, ranged from

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The predominant clinical picture in the literature is tinea corporis gladiatorum (TCG) [8, 9, 13–18]. More recently, Shiraki et al. [9] noted that the most common clinically infected body sites among judo practitioners and wrestlers were the trunk (55.4%), scalp (29.3%), and hands (1.1%). Notably, 30.4% of the athletes they examined were asymptomatic carriers. The reported prevalence rates of TCG ranged from 20–75% [14, 31]. In this investigation, the prevalence of dermatophyte infections was found to be 48.3%. The etiological agent of TCG cases has been reported to be Trichophyton tonsurans var. granulosum' (90.9%) and two zoophilic-type members of the T. mentagrophytes complex (referred to as 'T. rubrum var. granulosum') [33], and T. equinum [31] have been identified in some studies.

Recently, Ergin et al. [17] diagnosed symptomatic dermatophytosis in 29 of 32 wrestlers (90.6%), involving tinea capitis, tinea faciei, tinea corporis, tinea pedis and tinea unguium, in a boarding school in Turkey. The authors recovered a total of 22 isolates, i.e., 20 T. tonsurans (referred to as ‘T. tonsurans var. sulphureum’) isolates (90.9%) and two zoophilic-type members of the T. mentagrophytes complex (referred to as ‘T. mentagrophytes var. granulosum’) (9.1%). In this investigation, we also isolated T. tonsurans from samples of both scalp and glabrous skin, with and without lesions. To the best of our knowledge, our investigation describes the second outbreak of tinea capitis gladiatorum in Turkey, and the first to confirm that the outbreak was caused by the same genetic strain of this pathogen. However, we observed the ‘carrier state’ as the predominant clinical picture (Table 2). It is well established that asymptomatic carriage may play a
critical role in the transmission and persistence of dermatophyte fungi within a population [11,12].

In this investigation, 31.1% of the wrestlers were asymptomatic carriers of dermatophytes. This finding merits significant attention for those that participate in high contact sports events, such as our subjects, because (i) it may not be easy to identify an outbreak soon after the introduction of the pathogen into the population, (ii) clinical symptoms may only identify a minority of infected individuals, and (iii) symptom-free colonization of the scalp and/or trunk may easily trigger another dermatophyte infection and/or an outbreak, if not adequately managed. We also observed that TCS is almost always accompanied by ‘carrier state’ on the glabrous skin, e.g., trunk or inguinal area. It can be speculated that a wrestler with an infected scalp transmitted the dermatophyte fungi to his trunk or to the other athletes, as well as, a trunk carrier infecting his scalp. We believed that the former hypothesis is more probable, hence scalp ringworm is a major reservoir for the ‘carrier state’ of glabrous skin.

Monitoring of spore load may provide valuable information as to which individuals will remain carriers, develop symptomatic disease or clear their infection [11]. It is interesting (although not surprising) that none of the infected children had a heavy spore load, i.e., >10 colonies (Table 2). Hence, higher inocula levels may be required to cause symptomatic disease. In a recent study, we found four scalp carriers who spontaneously cleared the pathogen between 7–24 weeks without any antifungal therapy [23]. In the current investigation, we achieved mycological clearance after four weeks of terbinafine treatment. In contrast, our recent evaluation of griseofulvin suggested that less than one-half of treated carriers effectively cleared the pathogen [34]. Consequently, the best strategy for eradicating T. tonsurans in asymptomatic carriers remains unclear.

Our laboratory has accumulated extensive data on both symptomatic scalp ringworm and asymptomatic scalp carriage in Adana, Turkey [23,24,35,36]. Notably, T. tonsurans was only sporadically implicated in these cases. The emergence of this pathogen in countries where it is not endemic is on the rise but the mechanisms causing its spread are still speculative and the reasons for the increase in incidence are as of yet unclear [6]. It also remains to be elucidated whether the newly reported outbreaks of anthropophilic species have arisen from novel strains that have been recently introduced into the region or by strains that can be identified at low rates in the general population [37]. Hay et al. [6] noted that there is evidence in some cities in the UK that these organisms may have been present in the community for many years.

The finding of the same genetic profile in each of the isolates supports the suggestion that the infection pattern observed among this group of wrestlers represents a clonal outbreak rather than one of randomly acquired infections (Table 1). However, confirmation would require an understanding of the degree of genetic variability within the population of T. tonsurans isolates recovered from this geographic region, which is now unavailable. When compared with isolates recovered from various regions around the world, the strain observed in our wrestlers from Turkey has also been described among isolates recovered from the United States, Canada, and Japan [29]. Notably, all of the Japanese isolates derived from combat sports athletes which share this genetic profile are believed to have been introduced into Japan during international competition. Our wrestlers had not traveled abroad nor had they participated in any international competition. However, they may have wrestled other national athletes who could have visited abroad.

The detection of pathogenic fungi in non-living sites can provide valuable evidence as to the route of transmission [38]. Mackenzie [39] observed that T. tonsurans had almost certainly been present in the school included in his studies for 4 years prior to the investigation. The total number of affected children after a 4-year period represented only 16.4% of the population of the school, and it was suggested that T. tonsurans possesses a comparatively low degree of infectivity. Interestingly, El Fari et al. [16] identified T. tonsurans in cultures inoculated with samples from mats, one of the possible routes of infection, as in our study. We also isolated T. tonsurans from two pillowcases and one sheet. It is important to note that dermatophyte infection and/or re-infection may result from exposure to personal belongings and living areas.

Moisture, sweating, abrasion, and shared towels, clothing and showers may contribute to the increased incidence of cutaneous infections in sports medicine [8,17]. The most important control measure is regular inspection of each wrestler before practices and the prohibition of infected athletes from wrestling. Attention to hygiene practices (e.g., showering after practice, laundering clothes daily, disinfect wrestling mats) may decrease the transmission of dermatophytic fungi [30].

Shiraki et al. [8] identified risk factors for becoming a carrier or developing active infection during the sporting season. These included a history of scalp and neck involvement, failure to wear headgear, and failure to launder clothes at least once a week. In the current investigation, we identified that immediately prior to the outbreak, bedding had not been washed for 45 days as the housekeeper of the dormitory was out on maternity leave. She noted that her general practice was to wash whites at 70°C and 50°C for colors every 14 days. We also observed that the majority of our study’s wrestlers failed to wear headgear. In contrast, the coaches stated that the wrestling mats had been cleaned with diluted sodium hypochlorite solution after every training practice.
Results of this outbreak suggest that even though the number of cases was small, the 'carrier state', in particular scalp carriage, is more common than TCS cases (31.1% vs. 17.2%). It is important to be cognizant of this finding as untreated carriers remain a vector for spread to their team mates and they are also susceptible to developing overt clinical infection. In addition, TCS is almost always accompanied by carrier state of glabrous skin, screening inanimate objects is very informative in controlling such an outbreak, and molecular biological tools are necessary to demonstrate epidemiological relatedness of the T. tonsurans strains.

Acknowledgement
This work was supported in part by a grant from the Kenneth L. & Eva S. Smith Foundation.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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This paper was first published online on Early Online on 1 October 2009.