An epidemiological survey on cattle ringworm in major dairy farms of Mashhad city, Eastern Iran

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ABSTRACT

Background and Objectives: Cattle dermatophytosis (syn. cattle ringworm), an important skin infection, has received major consideration not only for economical losses in the animal breeding industry but also in regards to its zoonotic transmission to humans. For effective control measures, it is important to determine the disease prevalence in cattle herds.

Materials and Methods: To determine ringworm prevalence, a total number of 3,540 cattle in different age groups at three major farms of Mashhad including Kenebist (KB) in the east, Mazraeh Nemnooneh (MN) in the south, and Moghoofat Malek (MM) in the north-east were examined. Skin scrapings were prepared from all animals clinically suspected to have dermatophytosis. The samples were examined microscopically for fungal elements (hyphae and/or arthrospores) by adding potassium hydroxide (KOH) to samples. To isolate the etiologic dermatophytes, all samples were cultured on selective agar for pathogenic fungi medium for 4 weeks at room temperature.

Results: Among 684 suspected cases (19.3%) selected from a total number of 3,540 cattle based on clinical signs, 604 cases (88.3%) were KOH positive in direct microscopy, while 490 cases (71.6%) were culture positive on selective agar for pathogenic fungi (SAPF) medium. The most frequent dermatophyte isolated was Trichophyton verrucosum (495 isolates accounting for 99% of total isolates) which was obtained from all culture positive cases except five cases (1.0%) infected with another dermatophyte named Trichophyton mentagrophytes.

Conclusion: This work is the first comprehensive study on cattle ringworm in Iran. With respect to the high prevalence of cattle ringworm, particularly in young animals reported in the present study, effective management programs such as vaccination and improved hygiene are necessary for disease control in the herds.

Keywords: Dermatophytosis, Cattle, Trichophyton verrucosum, Trichophyton mentagrophytes, Iran.

INTRODUCTION

Dermatophytosis (syn. ringworm, tinea) is a zoonotic skin infection of keratinized tissues caused by a specialized group of fungi named dermatophytes. The disease has worldwide distribution and it has been considered as a public health problem all over the world (1). Animal dermatophytosis is responsible for high economical losses especially in cattle farming due to skin damages and decrease in milk and meat production.

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Dermatophytes include geophilic, anthropophilic and zoophilic species living in soil, human beings and animals respectively (2). For each animal species, the dermatophytes involved depend on the host studied and on the geographical and environmental conditions. Trichophyton verrucosum is the usual zoophilic dermatophyte involved in cattle ringworm throughout the temperate regions of the world. It also affects, but with lower prevalence, sheep, goat and other ruminants (3,4). The presence of T. verrucosum in the hair coat of free-ranging animals, especially non-ruminants, is uncommon. The animal age and trauma are important predisposing factors of disease (5).

Cattle ringworm mainly occurs in young animals (calves) and is rapidly spread in the herd via infected propagules, i.e. hyphae, and specialized fungal spores.
named arthrospores. The disease occurs worldwide and T. verrucosum is the almost exclusive etiologic agent (6,7). Besides cattle, it has been reported as the major agent of dermatophytosis in ruminants such as goat, sheep and camel (3,4,8,9). Aside from animal involvement, several human outbreaks of T. verrucosum infection have been reported so far by direct contact with infected animals or indirect contact with infectious propagules in the environment (10-12). Although various parts of human body may be involve by the fungus, the face and the body are mainly affected during the fungal infection. Human cases of T. verrucosum infection have been successfully treated by different antifungal agents such as azoles, but therapy for cattle is more difficult (12). Some reports support the efficacy of vaccination and improved hygiene for management of cattle ringworm in affected herds (7, 13, 14).

Despite the existing data about different clinical features of human dermatophytosis in Iran (15-18), little has been documented about animal involvement to date. In the present study, the prevalence and distribution of cattle ringworm in three major farms of cattle breeding in Mashhad were evaluated with special reference to etiologic dermatophytes.

### MATERIALS AND METHODS

#### Animals and sample preparation

Three major dairy farms of Mashhad located in different geographic areas including Mazraeh Nemooneh (MN) in the south, Kenebist (KB) in the east and Moghoofat Malek (MM) in the north-east were involved in this study during the Summer of 2004. All animals (calves and cattle; Holstein breed) in different age groups (3-6 months (1,544 cases), 7-12 months (641 cases) and >12 months (1,355 cases)) were examined. The animals were housed indoors at the time of sampling. The entire body of each animal was thoroughly examined for evidences of scaling, crusting, hair loss and erythema. Clinical lesions consistent with dermatophytosis observed in 684 of 3,540 animals were cleansed with cotton swabs soaked in 70% ethanol and then scrapped in the peripheral area with a sterile scalpel. Some of the animals which had no obvious lesions were also randomly sampled. The skin scrapings were collected in sterile petri-dishes labelled with the main animal characteristics and transferred to the laboratory for examination with standard methods.

#### Direct microscopic examination (DME)

The prevalence and distribution of cattle ringworm in major dairy farms of Mashhad city in East Iran with special reference to direct examination and culture results.

<table>
<thead>
<tr>
<th>Farm</th>
<th>No. of animals with clinical sings (%)</th>
<th>Age in month (No.)</th>
<th>No. of KOH positive cases</th>
<th>No. of culture positive cases</th>
<th>Fungal isolates (No.)</th>
<th>Total number of animals in each farm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kenebist (KB)</td>
<td>207 (16.9)</td>
<td>3-6 (137)</td>
<td>125</td>
<td>105</td>
<td>T. mentagrophytes (2)</td>
<td>535</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7-12 (65)</td>
<td>52</td>
<td>36</td>
<td>T. verrucosum (103)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>&gt;12 (5)</td>
<td>5</td>
<td>3</td>
<td>T. verrucosum (36)</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td>T. verrucosum (3)</td>
<td></td>
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<tr>
<td>Mazraeye Nemooneh (MN)</td>
<td>295 (19.7)</td>
<td>3-6 (236)</td>
<td>224</td>
<td>178</td>
<td>T. verrucosum (178)</td>
<td>703</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7-12 (52)</td>
<td>29</td>
<td>29</td>
<td>T. verrucosum (29)</td>
<td>1500</td>
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<tr>
<td></td>
<td></td>
<td>&gt;12 (7)</td>
<td>6</td>
<td>4</td>
<td>T. verrucosum (4)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>T. verrucosum (4)</td>
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<tr>
<td></td>
<td></td>
<td>3-6 (153)</td>
<td>138</td>
<td>117</td>
<td>T. mentagrophytes (3)</td>
<td>306</td>
</tr>
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<td></td>
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<td></td>
<td>T. verrucosum (114)</td>
<td></td>
</tr>
<tr>
<td>Moghoofat Malek (MM)</td>
<td>182 (22.3)</td>
<td>7-12 (25)</td>
<td>21</td>
<td>14</td>
<td>T. verrucosum (14)</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;12 (4)</td>
<td>4</td>
<td>4</td>
<td>T. verrucosum (4)</td>
<td>439</td>
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Table 1. The prevalence and distribution of cattle ringworm in major dairy farms of Mashhad city in East Iran with special reference to direct examination and culture results.
microscopic examination, a portion of each sample was placed on a clean glass slide and then covered with a clean glass cover-slip after adding a drop of 20% potassium hydroxide solution. The slides were gently heated over a flame for 1 minute and then examined for the presence of arthrospores and hyphae under a light microscope.

**Dermatophyte isolation and identification.**
Selective agar for pathogenic fungi medium (SAPF; E. Merck, Germany) containing soymeal peptone, 10.0 gr; D(+) glucose, 10.0 gr; cycloheximide, 0.4 gr; chloramphenicol, 0.05 gr; agar, 12.5 gr and 1 litre distilled water was used. Skin scrapings were inoculated on the SAPF medium prepared in plastic petri-dishes (8.0 cm diameter). The culture plates were then incubated at ambient temperature (~ 27ºC) for up to 4 weeks before discarding to ensure about the appearance of slow growing dermatophytes. The dermatophyte identification was made based on the colony characteristics and microscopic features of the fungal isolates according to the methods described in mycological textbooks (19-21).

**Statistical analyses.** The data from all three farms examined were subjected to statistical analysis using an under-windows SPSS Version 10.0 programme.

**RESULTS**
As demonstrated in Table 1, out of a total number of 3,540 cattle in different age groups from three farms in Mashhad, 684 cases showed clinical signs suspected to be dermatophytosis on visual inspection of various areas of animal body. These cases were from the farms KB (207 cases), MN (295 cases) and MM (182 cases). The major lesions observed on the affected calves and cattle were circumscribed, thick, hairless, gray-white skin patches affecting the head and neck. Among 684 suspected cases, 604 cases were KOH positive for fungal elements on DME out of which 490 cases were culture positive on SAPF agar. The remaining 80 cases were KOH negative and they did not yield any fungal growth on SAPF agar as well. All culture positive cases were KOH positive, while some of the KOH positive cases were reported to be culture negative as indicated in Table 1. All the randomly examined animals lacking the clinical signs consistent with dermatophytosis were both KOH and culture negative.

The frequency of disease in animals with lesions suspected to be dermatophytosis varied considerably in different age groups. In all 3 farms, the highest frequency was reported for the 3-6 months age group followed by the 7-12 months and >12 months age group (Fig. 1). For the KB farm, the frequency of disease was 23.4%, 18.9% and 1.2% for the above age groups respectively, while it was reported as 25.3%, 9.9% and 1.2% for the MN farm and 45.1%, 29.2% and 0.9% for the MM farm (Fig. 1). The only significant difference in disease prevalence was reported for the 3-6 months and 7-12 months age groups in the MM farm compared with the KB and the MN farms. Total frequency of disease for KB, MN and MM farms was reported as 14.9% (182 of 1,223 cases), 17.3% (259 of 1,500 cases) and 19.9% (163 of 817 cases) respectively (Fig. 1).

Concerning the fungal species responsible for cattle dermatophytosis, *T. verrucosum* was isolated from all cases except 5 animals in the 3-6 months age group on the KB (2 cases) and the MM (3 cases) farms which were infected with another dermatophyte named *T. mentagrophytes* (Table 1). In total, *T. verrucosum* was isolated from culture positive samples of the age groups 3-6 months (395 isolates), 7-12 months (79 isolates) and >12 months (11 isolates). Morphological features of an isolate of *T. verrucosum* is shown in Fig. 2. On SAPF agar, colonies were small, button or disk shaped, white to cream colored, with a suede-like to velvety surface, a raised centre, and flat periphery with some submerged growth (Fig. 2A). Microscopically, broad, irregular hyphae with many terminal and intercalary chlamydomspores were present in slide cultures prepared from the fungal colonies (Fig. 2B). The chains of chlamydomspores referred to as “chains of pearls”, were a predominant microscopic feature of *T. verrucosum* as shown in Fig. 2B. These structures have diagnostic value for the fungus.

**DISCUSSION**
The results of this study clearly demonstrate that dermatophytosis is a major problem in cattle farms in
Eastern Iran especially in young animals. *T. verrucosum* usually affects cattle and calves, however, it has been reported as the major agent of disease in ruminants such as goat, sheep and camel (3, 4, 8, 9). Recently, *T. verrucosum* infection has been reported affecting a wild goat-like animal named chamois (*Rupicapra rupicapra*) from Italy which indicates the importance of disease in the wild environment (22). The human infections with *T. verrucosum* have generally been reported from Eastern Europe, Central and South America, and the Middle East (1). The fungus is able to survive in skin scales of infected animals for up to several months in moist and dark places where it can be easily transmitted to human and other animals (2). Several outbreaks of disease have been reported in cattle herds especially in young animals (6,7,11).

Many researchers reported the occurrence of various types of human dermatophytoses from different geographic regions of Iran (15-18). Various dermatophytes from all three major genera of *Trichophyton*, *Microsporum* and *Epidermophyton* were reported as the etiologic agents of disease out of which some involved zoophilic species such as *T. verrucosum* were found to be transmitted from infected cattle to humans. Despite the large volume of data on human dermatophytosis, very little has been documented in regards to the involvement of animals in Iran. According to the report of Khosravi and Mahmoudi in 1990s, several dermatophytes were isolated from skin scales of affected animals out of which *T. verrucosum* was reported as the exclusive agent of cattle ringworm (23).

Cattle breeding in Iran has received major considerations not only as a main source of meat and milk production, but also for its importance in the leather industry. Due to the lack of comprehensive studies on cattle ringworm in Iran, there are no reliable data about exact economical losses from disease annually. The unpublished reports from different geographic regions of Iran and a recent report on cattle ringworm due to *T. verrucosum* from Kermanshah Township (24) indicate that ringworm is an important problem in animal breeding especially in cattle herds.

In this investigation, the prevalence and distribution of cattle ringworm was studied in a large population of cattle and calves (3,540 animals) from the major farms of Mashhad as an important site for cattle breeding in Eastern Iran. The disease mainly affected young animals in the 3-6 months age group (31.5%) followed by the age groups 7-12 months (15.9%) and >12 months (1.1%). There was significant difference between the frequency of ringworm in calves (<12 month, 26.9%) and cattle (>12 month, 1.1%) from all three farms examined in this study. The lower frequency of ringworm in cattle compared to calves may be attributed to the development of cell-mediated immune response elicited by the *T. verrucosum*, which results in resistance in adults. Based on the total frequency of cattle ringworm in the KB, the MN and the MM farms, there was no significant difference in geographic distribution of disease in Mashhad. However, the higher frequency of disease in 3-6 months and 7-12 months age groups in the MM farm compared to the KB and the MN farms may be due to poor hygiene and high density cattle breeding in the MM farm. For all cattle and calves, the disease prevalence was 17.3% which is in accordance with the mean prevalence of cattle ringworm (20.0%) reported worldwide (21).

*T. verrucosum* is responsible for most cases of cattle ringworm (more than 90%) worldwide. However, other dermatophytes such as *T. mentagrophytes*, *M. gypseum* and *M. canis* have been reported as the less...
common agents of disease. In this study, from a total number of 490 dermatophyte isolates obtained from infected animals on examination at mentioned farms, 485 isolates were identified as *T. verrucosum*, while only 5 isolates were diagnosed as *T. mentagrophytes*. Kakepis *et al.* (25) reported the cattle ringworm with the sole etiology of *T. mentagrophytes* with a frequency of about 35% in seven cattle barns during 1976-1977. The zoophilic fungus, *T. mentagrophytes* isolated in this study may be associated with frequent contacts of the calves with rats and pet animals like dogs and cats as it was reported for cattle from traditional-type farms (26). A recent report indicated that the relative frequency of the isolation of *T. verrucosum* (cattle ringworm) has decreased by 90% during a 25 years period from 1980 to 2005 in United Kingdom (27). This may be a reflection of improving hygienic condition of people and use of effective programs for controlling the ringworm in cattle herds.

In regards to the correlation between the prevalence of ringworm and economic losses or public health hazards observed in the present study, different aspects of disease were noticed. Some of the workers who had direct contact with the infected animals showed involvement of the face area mainly as tinea barba (data not shown). These individuals were treated with common antifungal agents. On visual inspection, an obvious reduction in weight gain was seen in some affected calves compared with unaffected animals of similar age. As indicated by the farmers, milk production and the quality of leather was also affected in heavily infected dairy cattle in all three farms examined. Despite the above-mentioned economical losses in cattle breeding farms, real losses to the cattle breeding industry in Iran is not possible to calculate due to lack of informative and documented data from different parts of the country.

In conclusion, results of the present study clearly highlight the importance of cattle ringworm in Iran. The high prevalence of disease seems to be associated with high density and poor hygienic conditions of animals in the herds. Routine evaluation of all cattle and calves accompanied with suitable control strategies, i.e. vaccination and improved hygiene, may be useful for managing ringworm as an economically important zoonotic infection.

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