Positive effects of attenuated *Trichophyton verrucosum* strain administration in treatment of the bovine trichophytosis

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SUMMARY

Trichophytosis is an endoctic skin disease caused by *Trichophyton* spp. In this study, the beneficial effects of 2 intramuscular injections (10 mL) of attenuated *T. verrucosum* strain (2.5 10⁶ agents/mL) at 2 weeks of interval on 9 dermatophytosis affected cows were investigated by evaluation of clinical recovery and measurement of biochemical markers of liver and kidney functions (enzyme AST, ALT and ALP activities and serum BUN, uric acid and creatinine concentrations, respectively) in blood serum collected on days 0 and 14 (before and 6 hours after the intramuscular injection) and on Day 42. Firstly, the presence of virulent *T. verrucosum* strain in skin lesions of all affected animals was confirmed by isolation and microbiological identification. Total or partial clinical recovery (diminution of the number and size of the skin lesions, reduction of the infected body areas, and disappearance of scurf, scales, hair loss, and keratinisation zones) was observed 4 weeks after the second injection in 66.6% (6/9) and in 33% (3/9) of affected cows respectively. Animals in which clinical recovery was incomplete were the most severely infected before the beginning of treatment. Furthermore, no significant alteration of liver and kidney functions was induced by administration of the attenuated *T. verrucosum* strain. Consequently, because of the significant reduction of the clinical signs and the absence of side effect, vaccination with attenuated strain of *T. verrucosum* may be beneficial in the treatment of bovine trichophytosis.

Keywords : Trichophytosis, cattle, *Trichophyton verrucosum*, attenuated strain, clinical recovery, skin lesions, biochemical parameters.

Introduction

Ringworm is a fungal infection of superficial, keratinized layers of epidermis and hair in animals [2, 16, 19]. *Trichophyton verrucosum* is a causative agent of bovine dermatophytosis and it brings on persistent dermatomycosis infections [18, 20, 31]. Individual differences, race predisposition and environmental conditions are important factors for the disease development. The mean incubation period is estimated at 4-6 weeks [10]. Spread of ringworm is probably mainly due to the licking and grooming activities of the calves tongues, and young, weak and immune suppressed animals may be predisposed [7]. In addition, fungal spores, animals contact and infecting materials are involved in the transmission of the disease. Sick or asymptomatic animals may contaminate other animals, humans and environment [15, 30]. Extensive alopecia and/or circumscribed thick hairless skin patches are observed on the head, neck, flanks and limbs. Characteristic locations for lesions are thorax and limbs for cows and heifers, ocular regions for young cattle, dewlap and inter-maxillary space for bulls [23, 31]. The lesions can persist for several weeks despite topical treatments with various anti-fungal drugs [31]. Detection of disease agent on pathologic materials, microscopic examination, isolation and identification with culture are used for the diagnosis of the disease [33].

Up till 1990’s some vaccines have been developed and/or applied in veterinary practice against ringworm in cattle, horses, and fur animals. All these preparations are monovalent alive...
vaccines which, however, have short storage life if they are not lyophilized. In addition, all these vaccines like attenuated strains are potentially dangerous for immuno-deficient individuals; they can revert to virulent forms and therefore may constitute some infection risk for human beings. In order to avoid all these disadvantages, a new sort of vaccine has been developed by the selection of highly immunogenic strain of *Trichophyton verrucosum* and inactivation under proper conditions [32]. Cell-wall antigens, as well as metabolites secreted by growing fungal cells, constitute the major antigens presented to the infected host. Vaccines, contained *Trichophyton verrucosum* strain stimulate a humoral and cellular immune response against these antigens [13]. The immune response conferred by the vaccine contained *Trichophyton verrucosum* strain measured by the delayed-hypersensitivity (DTH) and the migration inhibition test (MIT) is relatively intense and is chiefly of cellular type in guinea pigs and calves [13, 32]. This immune response emerges with an increase of the number of inflammatory cells include macrophages, lymphocytes and T cells in the dermis. The effector mechanisms involved in the elimination of dermatophytosis are not fully understood. However, several studies suggested that, phagocytosis and oxidative products of the immune response cells participated in the killing of the dermatophytes in vivo [13].

Some researchers reported that several systemic anti-fungal agents could bring adverse effects as mild to several hepatic injuries and renal syndromes. Therefore, some biochemical parameters (serum aspartate aminotransferase (AST), Alanine aminotransferase (ALT), and alkaline phosphatase (ALP) enzyme activities and blood urea nitrogen (BUN), uric acid, and creatinine concentrations) were used for detection and monitoring of hepatic and renal injury induced by anti-fungal drugs [6, 9, 14, 17]. AST is present in many tissues and body fluids and elevated serum activity is due to tissue damage, especially in heart and liver. Because ALT is mainly present in hepatocytes, increased serum ALT activity reflects liver injury. The ALP is an enzyme localized on cell membranes: serum ALP activity is elevated in hepatobiliary diseases, especially in obstructive jaundice. The blood (serum or plasma) urea concentration conventionally specified in terms of nitrogen content and called blood urea nitrogen (BUN), is an important indicator of renal function. Uric acid is the end product of purine metabolism or oxidation in the body. Increased serum uric acid concentration is an indicator of hepatic insufficiency. Creatinine is the terminal nitrogenous compound of the muscular creatine metabolism, which is eliminated in urine after renal filtration and elevation of blood creatinine concentrations indicates a disturbance in kidney function [5].

The aim of this study is to investigate the clinical recovery in cattle with dermatophytosis treated by attenuated *T. verrucosum* strain. The determination of possible adverse effects of this vaccine on liver and kidney functions of affected animals is also aimed.

### Materials and Methods

#### ANIMALS

Nine local dark race cows with Trichophytosis were investigated in this study. Cows were 1-6 years old (5 animals were 1 year old, 2 animals were 2 years old, 1 animal was 4 years old and 1 animal was 6 years old) and were managed in similar closed herds in Samsun province, Turkey. Housing conditions of the animals were closeness and poor ventilated.

### MICROBIOLOGICAL INVESTIGATIONS

For the laboratory diagnosis of Trichophytosis, the specimens of animal hair and skin were taken from the 9 affected cows. The animals were stemming from various barns. The foreign matters into the lesions, contaminating fungus spores and the other factors such as particles of powder, sand and hay were removed by cleaning the lesions with 70% alcohol. After alcohol dried, sufficient amount of skin scrapings and feather specimens were taken from the active parts of the lesions’ edges by the help of the sterile scissors, forceps, lancet or curette for direct microscopic examination, isolation and identification.

The sowings from the specimens were made by burying the pathological matters on the nutrition place’s surface with sterile forceps or lancet. The nutrition place was incubated in aerobic conditions at 25 °C and 37 °C for 3 weeks [3]. Macroscopic characteristics of colonies (reproducing situation and time, shape, structure and the pigmentation characteristics on the front and back surfaces) were recorded every day during the incubation period. For microscopic examination, preparations obtained from cultures and painted with lactophenol cotton blue were used. The preparations were evaluated for the structures of hifa, microconidium, macroconidium, clamidospore, artrospore and blastospore in genus rank [3, 21]. The detailed identification of the isolates was made according to the procedures described by REBELL and TAPLIN [25].

### EXPERIMENTAL DESIGN

Ten mL of attenuated strain of *T. verrucosum* containing 2.5x10^6 immunogenic agents per mL (Trichoben®, Biobeta A.S., Ivanovice na Hané Czech Republic) were injected to Trichophytosis affected animals (n = 9) by intramuscular route in the gluteal muscle two times (at Day 0 and Day 14). Before and after the 2 treatments with attenuated *T. verrucosum* strain, the numbers, spreads, forms and localisations of the lesions and scurf, hair loss, pruritus, scales, keratinisation and crust were scored.

On days 0 and 14, blood collection was realised 2 times; before the I.M. injection of attenuated *T. verrucosum* strain and 6h after. Furthermore, blood samples were collected again on Day 42 (4 weeks after the second injection) for determination of biochemical changes during this period. After blood clotting at 37 °C during ten minutes in an incubator, serum was isolated by centrifugation (10 minutes, 1550 g, at 4 °C) and stored at -20 °C until the assay. Serum AST, ALT, and ALP enzyme activities and blood urea nitrogen (BUN), uric acid, and creatinine concentrations were analysed by an auto-analyser (Autolab, The Netherlands). The analysis was performed with the commercial kits (Sigma-Aldrich Chemie GmbH, Germany) according to the manufacturer’s instructions.
EFFECTS OF ATTENUATED TRICHOPTHON VERRUCOSUM STRAIN IN TREATMENT OF THE BOVINE TRICHOPTHYSIS

STATISTICAL ANALYSIS

ANOVA one way and Duncan test were used for comparison of serum AST, ALT, ALP activities and BUN, uric acid and creatinine concentrations. Also, Wilcoxon test was used for evaluation of clinical score obtained in trichophytosis-affected cows before and 28 days after the last intramuscular injection of the attenuated strain of T. verrucosum [24]. All data were expressed as mean ± standard deviation. Differences were considered as significant when p values were less than 0.05.

Results

Trichophyton verrucosum was isolated and identified from all of the specimens stemming from the trichophytosis suspected animals (n = 9).

Before the treatment with attenuated T. verrucosum strain, skin lesions were generally annular except on 2 animals (cows no5 and 9) and were limited to the head and neck in 6 cows. In the 3 other animals, they also occurred in the flanks and limbs. Pruritus was absent in all animals. All cows presented hair loss zones. Scurf, scales, crusts and keratinisation areas were also generally noted in the affected cattle, but they were weak (scurf and hair loss) and even absent (scales, crusts and keratinisation areas) in 2 animals (cows no7 and 8) whereas they were remarkably severe in one animal (cow no9) (Table I). The average clinical score was 11.8 ± 4.5 and ranged between 4 (cow no7) and 19 (cow no9). Clinical signs were considerably alleviated 4 weeks after the 2 injections of the attenuated strain of T. verrucosum (Table I): 5 cows did not show any clinical signs of trichophytosis. On the other cows (the most affected previously), the number of lesions markedly decreased and scales, crusts and keratinisation areas have totally disappeared except on the cow no9. The reduction of scurf was also evidenced (except again on the cow n°9). Taken together, the mean clinical score (2.8 ± 4.5; extreme values 0 – 13) was significantly depressed (p<0.05). Serum enzyme (AST, ALT, ALP) activities and BUN, uric acid and creatinine concentrations remained relatively constant throughout the experimental period (6 weeks) (Table II) and applied treatment has not induced significant variations of any biochemical parameters in serum (p>0.05). No significant correlation between clinical score and each analysed biochemical parameters after treatment (on Day 42) was obtained. These findings confirmed the absence of adverse effects induced by the treatment with attenuated strain of T. verrucosum on liver and kidney functions.

Discussion

Ringworm infections in domestic animals are a major veterinary and public health problem [30, 31]. The most frequent causative agent of bovine dermatophytosis in many countries T. verrucosum, is a cosmopolitan, zoophilic dermatophyte fungus [2, 8, 18, 20]. This agent was also sometimes isolated from sheep, goats, horses, donkeys, dogs and camels [1, 4, 12, 22]. T. verrucosum is also a common cause of human ringworm and humans may be infected with the agent directly or indirectly from cattle [12]. As T. verrucosum was isolated from cows with no apparent skin lesion [30], dermatophytosis might be easily transmitted through contact with the infected calves [30].

<table>
<thead>
<tr>
<th>Cow</th>
<th>Number/localisation</th>
<th>Form</th>
<th>Characteristics of the skin lesions</th>
<th>Clinical score</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hair loss</td>
<td>Scurf</td>
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<tr>
<td></td>
<td>BT</td>
<td>AT</td>
<td>BT</td>
<td>AT</td>
</tr>
<tr>
<td>1</td>
<td>H  (1+)</td>
<td>0</td>
<td>A  (1+)</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>H  (1+)</td>
<td>0</td>
<td>A  (1+)</td>
<td>0</td>
</tr>
<tr>
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<td>H  (1+)</td>
<td>H  (1+)</td>
<td>A  (1+)</td>
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</tr>
<tr>
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<td>A  (1+)</td>
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<td>D  (2+)</td>
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<td>A  (1+)</td>
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</tr>
<tr>
<td>9</td>
<td>W  (5+)</td>
<td>H  (3+)</td>
<td>D  (2+)</td>
<td>A  (1+)</td>
</tr>
</tbody>
</table>

**Mean** 2+ 1+ 1+ 2+ 0 2+ 0 2+ 0 2+ 0 2+ 0 2+ 0 11.8 2.8

**Localisation**: H: Head; Fa: Face; N: Neck; F: Flanks ; L: Limbs; S: Sacral region; **Form**: A: Annular; D: Diffuse – **Others** (presence of scurf, scales, crusts and keratinisation areas): 3+: Severe; 2+: Moderate; 1+: Weak; 0: Absence. BT: Before treatment – AT: After treatment. Means with different superscript in the same line significantly differ (p<0.05).

**Table 1**: Clinical signs and clinical score obtained in trichophytosis-affected cows (n = 9) before and 28 days after the last intramuscular injection of the attenuated strain of T. verrucosum (the treatment consists in 2 intramuscular injections at 2 weeks of interval).
Biochemical parameters | 0h | Day 0* | 6h | 0h | Day 14* | 6h | Day 42
-- | -- | -- | -- | -- | -- | -- | --
AST (U/L)     | 128.0 ± 27.7 | 125.1 ± 30.1 | 122.9 ± 33.8 | 115.1 ± 26.2 | 120.3 ± 28.1 |     |     
ALT (U/L)     | 31.7 ± 8.9   | 28.3 ± 6.1   | 25.3 ± 5.6   | 25.2 ± 5.4   | 25.4 ± 4.2   |     |     
ALP (U/L)     | 152.1 ± 30.9 | 158.3 ± 31.6 | 155.6 ± 31.5 | 153.8 ± 24.4 | 144.7 ± 34.3 |     |     
BUN (mg/L)    | 251.0 ± 84.0 | 268.0 ± 77.0 | 243.0 ± 91.0 | 236.0 ± 69.0 | 281.0 ± 82.0 |     |     
Uric acid (mg/L) | 18 ± 5      | 17 ± 4       | 18 ± 3       | 17 ± 2       | 15 ± 4       |     |     
Creatinine (µmol/L) | 106.1 ± 44.2 | 114.9 ± 44.2 | 123.8 ± 44.2 | 114.9 ± 44.2 | 123.8 ± 53.0 |     |     

*On days 0 and 14, blood collection was realised 2 times; before the I.M. injection of attenuated \textit{T. verrucosum} strain and 6h after.

Table 2: Serum enzyme (AST, ALT, ALP) activities and substrate concentrations (BUN, uric acid and creatinine) in dermatophytosis affected cows (n = 9) treated by attenuated \textit{T. verrucosum} strain (the treatment consists in 2 intramuscular injections on Days 0 and 14) according to time.

Topical and systemic anti-fungal agents have been used for bovine trichophytosis. But, some studies indicated that clinical adverse effects could have been seen in therapy with various systemic anti-fungal agents. Several case reports of mild to severe hepatic injury, including icteric and fatal cases, have been published \[9, 17\]. For instance, adverse effects of griseofulvin are gastro-intestinal symptoms, allergic reaction, photo-dermatitis, hepatic and renal dysfunctions. Similarly, ketoconazole adverse effects are hepatic dysfunction or asymptomatic increases of serum transaminases activities \[9\]. In addition, nausea and vomiting are seen in itraconazole treatment \[14\]. By contrast, no severe hepatic injury, including icteric and fatal cases, have been published \[9, 17\]. For instance, adverse effects of griseofulvin therapy. However, efficient control of bovine ringworm with vaccine administration of a live vaccine against bovine trichophytosis has been reported by GUDDING and LUND \[13\]. Consequently, the \textit{T. verrucosum strain} had no negative effect on liver and kidney functions. As a conclusion, intramuscular injections of attenuated \textit{T. verrucosum} strain had no negative effect on liver and kidney functions. As a conclusion, intramuscular injections of attenuated \textit{T. verrucosum} strain efficiently cure or at least markedly attenuate in the most severe cases the bovine trichophytosis without inducing adverse systemic effects.

### References


TRICHOPHYTOSIS

EFFECTS OF ATTENUATED TRICHOPHYTON VERRUCOSUM STRAIN IN TREATMENT OF THE BOVINE TRICHOPHYTOSIS


