EXPERIMENTAL *Trichophytone Mentogrophytes Var Mentogrophytes* INFECTION IN RABBITS: CORRELATION BETWEEN IMMUNOLOGICAL AND CLINICAL OBSERVATION.

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ABSTRACT

To questing the severity and courses of experimental infection with *Trichophyton mentogrophytes var mentogrophytes* in four groups of eight rabbits, prepared HIS against the fungus was used in different routs and doses in two groups (second and third group). 0.2ml subcutaneously in 2nd group and0.1 ml interadermally in 3rd group. The shorter course of the infection was recorded in 1st group previously infected and left with out treatment with HIS, while complete healing was recoded after 5 weeks and 8 weeks in 2nd and 3rd group respectively in compared to 4th group (control group) after 10 weeks.

INTRODUCTION

Dermatophytosis, or ringworm or tinea is an infection of superficial keratinized structures of skin and hair of animals and human. The disease is caused by a group of keratinophylic filamentous fungi belonged to the genera *Trichophyton, Microsporum* and *Epidermophyton*. The dermatophytes are molds, characterized morphologically by hayphae forming a mycelium when grown on suitable media (1, 2, and 3). Ringworm denotes the clinical entity characterized by annular skin lesions. Although it is a superficial skin infection, ringworm in cattle may have a significant impact on management and economics in affected herds (2). The *T. mentogrophytes* and *T. verrucosum* were isolated from natural feedlot calves and sheep from different herds in Dohuk province\ Iraq and *T. mentogrophytes var mentogrophytes* was isolated from white rats at breeding laboratory home at veterinary medicine, Dohuk university .Iraq (4). Dermatophytosis also has important economic significance, as apparently healed skin

lesions reappear after tanning process (5). Consequently, a national vaccination program was advocated by the Swedish hide industry (6). In Norway and a few other countries, ringworm in cattle is a notifiable disease. Restriction on the sale of breeding animals and on the access of livestock to common pastures is imposed of affected herds (2).

Treatment of clinical ringworm in cattle is expensive and time consuming; there is definitely a need for effective prophylaxis against the disease as hygienic and other preventive measures often fail (2, 3).

MATERIALS AND METHODS

Preparation of Hyper immunoserum:

An adult rabbit was used for purpose of preparation of hyper immune serum (HIS). The preparation was done by given subcutaneously five successive 0.2ml doses at weekly interval of growth culture of *Trichophyton mentogrophytes var mentogrophytes* which suspended in buffer solution PH 7.4 and the collected serum after one week of last dose (after five week) was used in two groups as following.

Experimental infection:

The study was conducted in to nine adult rabbits. One of them was used for preparation of hyper immune serum and the others were grouped in to four groups each of two rabbits. The first group was experimentally exposed to infection with *Trichophyton mentogrophytes var mentogrophytes* before three months and spontaneously completely cures from natural primary infection and then re infected with the same species of fungus. The second group was treated with 0.2ml subcutaneously of hyper immune serum (HIS) and exposed to experimentally infection after 10 days of post treatment with the HIS. The third group was treated with 0.1ml intradermally of HIS and exposed to experimental infection with the same strain of the fungus immediately. The fourth group was kept as control group for infection.

RESULT

The site of I\d injection was showed severe hyperemic reaction after 12 hrs of inoculation (Trichophytin reaction) as shown in fig (1).

Fig (1) shows trichophytin reaction following 12 hours from intradermally injection of dermatophyte antigen.

The typical clinical signs of the disease were observed in all groups (1st, 2nd, 3rd and control group) within the 7 days after experimental infection which including the scaly type lesions with crust edges on a bleeding base covering more than 5 cm in diameter at inoculated area as fig(2).

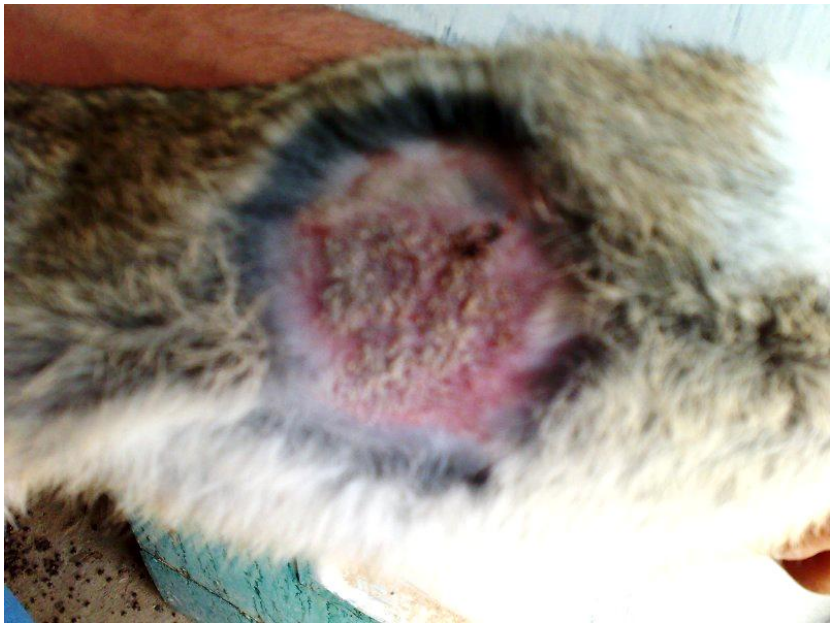

Fig (2). Scaly type lesions with crust edges on a bleeding base.

The courses of the infection were differed among groups. Complete healing was indicated after 3 wks in group one which was previously infected without HIS. And also healing revealed in group 2 after 5 wks of treatment with s\c of HIS. While in the group 3 which treated with 0.1ml I\d of HIS after 8 wks. And the spontaneously healing was observed after 10 wks in control group.

DISCUSSION

The dermatophytes, like other fungi, have a very complicated antigenic makeup. Antigenic substances of the dermatophytes are glycopeptides, peptides, or carbohydrates, and the individual types of antigens may elicit different types of responses. In addition, the keratinase of *Trichophyton mentagrophytes* are an important antigen not only because it elicits strong delayed hypersensitivity responses but also because antibodies that inhibit the proteolytic activity of this enzyme are produced (7) Debeor *et al* (8) showed in five of previously infected cats with *M.canis* and only two of 10 control had immediate positive reactions to *M.canis* antigen injected intrademally and after 24 hours after injection , four out of five previously infected cats had delayed reaction at the injected sites, equivalent sites in all control animals were negative. Grapple *et al* (9) showed the immunity was apparent 7 to 9 days postinoculation and persist for 18 month. Kaaman and Mohamed (10, 11). Found that the cellular response in vaccinated animals can be demonstrated by intrademal injection of the trichophytin.

In the present study revealed severe trichophytin reaction these due to infiltration of cluster of neutrophils at the inoculated sites and many of them may degenerate this in agreement with similar finding by (12). Kerbs *et al* (13) showed guinea pigs which inoculated with *T.mentogrophytes* with positive reactions intradermic test with trichphytin between the 7^{th} and 9^{th} days after inoculation. Woodfolk and Platis (14) mentioned the rule of the neutrophils in the defense mechanisms is not totally clear, it has been demonstrated in experimental models that neutrophils infiltration occur before peak of infection. Where as David *et al* (15) showed the cell wall of the dermatophytes is made up primarily of chitin and glucan in addition to the glycopeptides which represent the major antigens of these organisms.

The present study also recorded typical signs of the disease in all groups within 7 days of the experimental infection which characterized by scaly type lesions with crusty edges such signs were also found by (16). As well as the severity and immunological reaction differ with groups , severe reaction was found in group (1,2) with less severe reaction was in groups

(3,4). This due to memory cells in the healed skin at the site of a first infection have been demonstrated after experimental *Trichophyton mentagrophytes* infectious in guinea pigs (17). In cattle, a previous infection appears to afford partial immunity to a second infection at the same site as well as at different sites (18). A state of relative acquired resistance to re infected with the same or another species of dermatophytes may results from cutaneous infection, this resistance may vary in both degree and duration, depending upon several factors including the species or strain of dermatophytes (zoophilic or anthrophilic) the host (animal or human) and the site of infection (smooth, skin, hair or nail) (9).

On the other hand the courses of the disease were also recorded in this study and revealed shorter course in group one about 3 weeks and the longer course about 10 weeks in group four. Whereas grapple *et al* (9) reported in rabbit and cat the course of the second infection resembled that of the first one and no evidence was obtained for immunity or cutaneous sensitization after one infection. Sparkes *et al* (19) showed the lesions of ringworm in four of five cats that infected experimentally by topical application of mycelium to shaved abraded skin remain stable for between 1 and 6 weeks with clinical resolution evident by 11-14 weeks post infection , while in one cat disease was prolonged at the end of the 16 week from inoculation. Elad and Segal (20) reported decrease in the course of clinical signs from 9.5 weeks to 3.7 weeks in calves immunized with crude ribosomal fraction (CRF) subcutaneously with two injection of two week interval. Naess and Sandvik (21) and Liven and Stenwig (22) reported in trial in group of non infected animals vaccinated and kept together with non vaccinated animals and are subsequently exposed to natural infection by introduction of a calf with clinical trichophytosis seem to be challenge model that best approaches the natural disease in such experiment with live vaccine, clinical lesions from which *T. verrucosum* could be re isolated in un vaccinated animals 3 to 4 weeks post challenge, where as no enduring lesions were observed in the vaccinated animals.

الإصابة التجريبية لفطر

Trichophyton mentogrophytes var mentogrophytes

في الأرانب: دراسة العلاقة بين المناعة والعلامات السريرية.

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الخلاصة

استهدفت هذا الدراسة لمعرفة الضراوة و فترة العدوى التجريبيةِ لفطر *Trichophyton mentogrophytes var mentogrophytes* في ثمانية أرانب مقسمة الى اربعة مجاميع، استخدمت المصل المنيع المهيء ضدّ الفطرِ بجرعة 0.2 مليلتر تحت الجلد في المجموعةِ الثانيةِ و بجرعة 0.1 مليلترِ في الادمة في المجموعةِ الثالثةِ. اما المجموعة الاولى كانت مصابة سابقا بنفس عترة الفطرو شفيت تلقائيا بدون اعطاء المصل المنيع، اما المجموعة الرابعة استخدمت كمجموعة السيطرة.حيث لوحظ شفاء فترة الاصابة قصيرة جدا فى المجموة الاولى اما في الحيوانات المجموعة الثانية بعد 5 اسابيع و 8 اسابيع في المجموعة الثانية و الثالثة على التوالي مقارنة مع المجموعة السيطرة بعد10 اسابيع.

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