

IVERMECTIN ACTIVITY IN TREATMENT OF CATTLE DERMATOPHYTOSIS.

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ABSTRACT

In order to determine the dermatophytosis infection in cattle, twenty scraping samples were collected from cattle showed clinical signs of skin disease in different areas in Diyala governorate during a period from November 2012 to May 2013.

Results in cattle samples showed that 18 out of 20(90%) were positive for fungal infection. The highest percentage of infection was seen in November (50%), (30%) in February, and the lowest in March (20%). The main fungal species that isolated was *Trichophyton verrucosum*. A single subcutaneous injection of ivermectin at a dose of 200 microgram/kg. was administered to the affected animals. Recovery was observed after 10 days after injection, started by scales dropping and appearance of the hair. These findings show that ivermectin can be used successfully in the treatment of dermatophytosis in bovine induced by immunopotentiality due to the elevation in the total white blood cells (lymphocytes) after ivermectin injection.

Keywords: Cattle, dermatophytosis, ringworm , ivermectin

INTRODUCTION

Dermatophytosis (ringworm) 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 (Kane *et al.*,1997).Animal dermatophytosis is responsible for high economical losses especially in cattle farming due to skin damages and decrease in milk and meat production (Weitzman and Summerbell, (1995).

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The animal age and trauma are important predisposing factors of disease (Oborilova and Rybnikar,2005). Cattle ringworm mainly occurs in young animals (calves) and is rapidly spread in the herd via infected propagates, example hyphae, and specialized fungal spores named arthrospores. The disease occurs worldwide and *T. verrucosum* is the almost exclusive etiologic agent (Pier *et al.*,1994; Weber,2000) .Spores may survive in the environment for 2 to 3 years (Gudding and Lund,1995). Besides cattle, it has been reported as the major agent of dermatophytosis in ruminants such as goat, sheep and camel (Stenwig,1985; Pier *et al.*,1994; Fadlelmula *et al.*,1994). 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 propagates in the environment and also be spread to the hands of handlers (Ming *et al.*,2006; Scott,2007).

Human cases of *T. verrucosum* infection have been successfully treated by different antifungal agents such as azole compounds , but therapy for cattle is more difficult (Ming *et al.*,2006). It has been reported that animals housed in close proximity to each other for long periods and the presence of infected debris in buildings considered as the main causes of the infection(Dehghan *et al.*,2009). The initial lesions are discrete, grayish-white, crusty dry areas with a few brittle hairs . Some areas may become suppurative and thickly crusted. Lesions resembling light brown scabs may also be seen; when these scabs fall off, they leave an area of alopecia. The lesions usually resolve spontaneously in 2 to 4 months(Acha and Szyfres,2003). However, limited studies on cattle ringworm have been published in Iraq and the disease is considered to be common in most dairy farms of our country. The objective which be selected in this study is ivermectin.

Ivermectin is macrocyclic lactones are products or chemical derivatives of soil microorganisms belonging to the *Streptomyces avermitilis* fungus manufactured by different companies for drugs production as Saudi Pharmaceutical Company. The main uses of ivermectin in treatment of intestinal helminthes infections as strongyloidiasis , onchocerciasis and heart worm ,also it is an active agents in treatment of ectoparasites like ticks and lice (Yates and Wolstenholme,2004).

Therefore the aims of the present study are to determine the prevalence of cattle ringworm in native dairy farms of Diyala governorate, and the distribution of *T. verrucosum* and treatment of the infection in the bovine by ivermectin.

MATERIALS AND METHODS

1. Collection of Samples

Twenty samples were collected from cattle showed clinical signs of skin disease, for the isolation and identification of the causative agent. The shape, size, position, distribution and time of the appearance of skin lesions as well as the age of the animals were recorded. The lesions in affected animals were first rubbed with a cotton swab impregnated with 70% ethyl alcohol to remove surface adhering microorganisms and then skin scales were collected by scraping of the lesion using a sterile scalpel. These scraping samples were taken from the peripheral or edge of the lesion. The skin scrapings were collected into sterile Petri dish and transmitted to the laboratory under aseptic conditions (Cheesbrough,1992).

2.Direct microscopic examination:

The specimens were treated with 10% KOH to dissolve tissue material, leaving the alkali-resistant fungi intact, and stained with special fungal stains(Siegmund *et al.*,1979).

3. Sabouraud's Dextrose Agar (SDA) preparation:

The medium was prepared according to the manufacturer directions by dissolving 65 gm of the medium in 1000 ml of distilled water, chloramphenicol 0.05 gm/l of medium was added. After autoclaving at 15 lb/inch² pressure/121 C° for 15 minutes, the medium temperature was lowered to 50C° , so the cyclohexamide was added as anti saprophytic fungi then the medium was poured into sterile Petri-dishes for isolation or kept in slant screw capped bottles (universals) for maintaining the isolates.

4. Culturing of samples

Each sample was cultured directly on tow Sabouraud Dextrose Agar media which incubated in the incubator at 30C° to assist growth of moulds for 1-4 weeks before discarding to ensure the appearance of slow growing dermatophytes, with intermittent observation of the fungal growth and when the growth appeared and completed the identification test was done.

5. Identification of fungi

A. Optical examination of the colony :

In this test the colonies morphology, shape, color, consistency, texture and reverse plate color and other apparent characteristics were examined.

B. Microscopic Examination of the culture:

One drop of lactophenol cotton blue stain was put on the slide and then mixed with part of the colony by using sterile forceps, then covered with a

cover slide and examined under 40X lens to determine the shape of mycelium and spores(macro, microspores) and Chlamydo spores.

6. Ivermectin 1% : Manufactured by Saudi Pharmaceutical Company.

7- Animals: Forty infected animals were divided into two groups :

Group - A : In this group (study group) twenty animals (of different age and sex) were treated at a dose of 200 micrograms /Kg. B . Wt. by subcutaneous injection (Bogan and McKellar,2000). The treatment depend upon the clinical signs . The dose is repeated after 11 days in severe cases according to the information which had been taken from the manufacture corporation. Five milliliters of blood was be collected before and after the treatment from all the animals and they are reserves in EDTA vials to determinate the whole and differential white blood cells count. From recovered animal , a further five milliliters of blood has been taken to determinate the whole and differential white blood cells count .

Group - B : In this group (control group) twenty animals (of different age and sex) were treated by 1% sulpher ointment by local application once daily.

STATISTICAL ANALYSIS

The differences are compared by using (F-Test) at $p < 0.05$ (Zar,1984).

RESULTS AND DISCUSSION

The dermatophytes , a group of septate fungi which occur worldwide , invade superficial keratinized structures such as skin , hair and claws. The figure (1) reveals the shape of the lesions in the hand of the owner.



Figure 1: Reveals the shape of the lesions in the hand of the owner.

The figure (2) was revealed the arrangement of arthrospores on hair shafts.

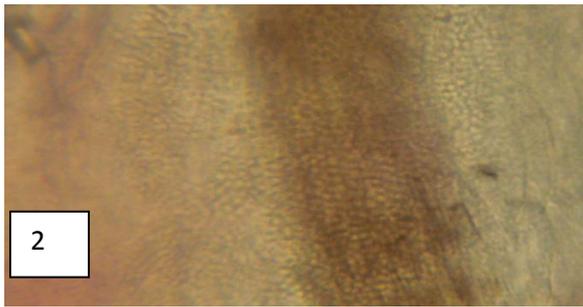


Figure 2: Reveals the arrangement of arthrospores on hair shafts.

The figure(3) reveals the shape completely grew colony of *T. verrucosum* on (SDA).



Figure 3: Reveals the shape completely grow colony of *Trichophyton verrucosum* fungi on (SDA) at 37C°.

The figure (4) was revealed the chlamydospores of *Trichophyton verrucosum* in chains.

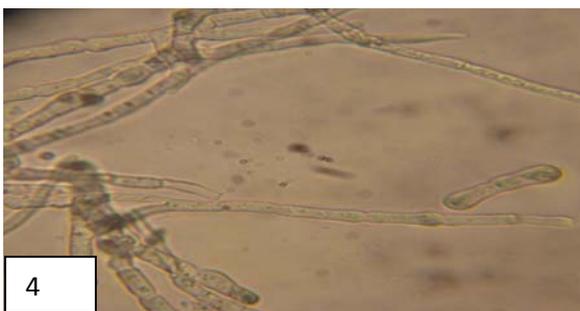


Figure 4: Reveals the chlamydospores of *Trichophyton verrucosum* in chain.

Table 1: Reveals the concentration and type of drugs used and the time of infection clearance.

Animal group	Drug concentration (1%)	Period of clearance Days
Study group	Ivermectin	13-24 *
Control group	sulpher ointment	30-40

* (P<0.05)

Table 2: Total number of the infected cattle, total and differential white blood cells count (Mean \pm Standard Error) determinate before, after treatment and after clearance of the lesions.

The parameters	Before treatment \pm S.E.	After treatment \pm S.E.	After recovery \pm S.E.
Total WBC in mm ³	5.390 \pm 1.025	6.490* \pm 0.422	6.130 \pm 0.587
Neutrophils (%)	59.300 \pm 4.250	45.100* \pm 9.560	54.300 \pm 0.380
Lymphocytes(%)	34.500 \pm 3.200	51.200* \pm 10.830	40.200 \pm 2.410
Monocytes (%)	4.300 \pm 1.400	2.200 \pm 1.152	4.500 \pm 0.832
Eosinophils (%)	2.400 \pm 0.422	1.500 \pm 1.092	1.100 \pm 0.224

* (P<0.05)

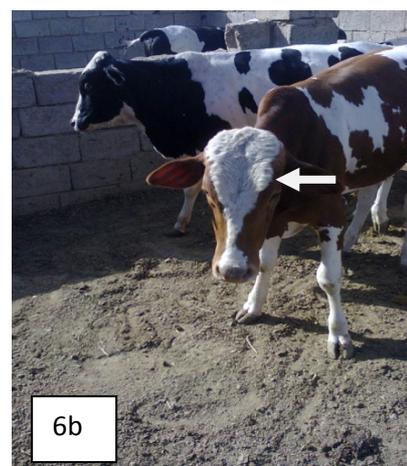
Sabouraud dextrose agar (PH 6.9) with the addition of 0.05g/liter chloramphenicol and 0.4g/liter cycloheximide. Inoculated plates are incubated aerobically at 37C° and examined twice weekly for up to 5 weeks. Most of the isolates are revealed the presence of *T. verrucosum* chlamydospores in chain ; rare macroconidia after culturing and examination. These results confirm *T. verrucosum* is the cause of ringworm in cattle in agreement with previous studies (Al-Ani *et al.*,2002; Cam *et al.*,2007; Quinn *et al.*,2011; Levinson,2012). Ellis *et al.*(2007) demonstrates , that *T. verrucosum* is the cause of ringworm in cattle, also refers to the infections in humans, result from direct contact with infected cattle or infected fomites and is usually highly inflammatory involving the scalp, beard or exposed areas of the body. Invaded hairs show an ectothrix infection. So preferable treatment of this infection is systemic therapy , with good response.

Statistical analysis reveals significant difference (p<0.05) when use the ivermectin in treating the disease with less time reach to 13-24 days shown in Table(1) compared with topical antifungal 1% sulpher ointment which needs more time for clearance 30-40 days and needs high cost(Radostits *et*

al.,2000).The figures 5a,b and 6a,b reveals the efficacy of ivermectin in treatment of ringworm disease in cattle with clearance rate of 100%.



Figures 5a,b : Reveals the lesion of ringworm disease before and after the treatment respectively.



Figures 6a,b : Reveals the lesion of ringworm disease before and after the treatment respectively.

The mode of action of ivermectin may be due to the stimulation of immune response particularly the cellular immune response of the animal and this effect is elicited from the results in Table -2 which shows significant elevation ($P < 0.05$) in the rate of lymphocyte cells after treatment by Ivermectin, so the mean values raised in all animals from 34.500 ± 3.200 % before treatment and reaches to 51.200 ± 3.830 % and this elevation accompanied by significant elevation ($P < 0.05$) in total white blood cells counts, so the mean values raised in all animals from 5.390 ± 1.052 % thousand cells before treatment and reaches to 6.490 ± 0.422 % thousand cells after treatment. There is no statistical signification ($P < 0.05$) in values of neutrophils and eosinophils before and after

treatment. The two elevations in total count of white blood cells and in percentage of lymphocyte cells were be share in raising the immunity of the infected animals. This result was supported by previous study was done by Pier *etal.*(1993) which were refer that a combination of cell-mediated and humoral responses is required for immunity and clearance of *T.verrucosum* infection.

This study agree with Kirmizigul *et al.*(2012) which were referred to the ability of ivermectin in treatment of ringworm disease in cattle with 90% clearance rate at a dose of 200 micrograms /Kg. B . Wt . Also this study was agree with Sajid *et al.*(2007) who refer to immunopotentiating effect of ivermectin on treated animals when the dose was duplicated because, the potent cause which interfere with disease cure is the good immunity . Other studies were referred to the role of cellular branch of immune system in protective immunity against dermatophyte infections(Kaaman,1985; Sohnle, 1993). Blakley and Rousseaux, (1991) disclosed that antibody production and T-lymphocytes and macrophages dependent response were enhanced after ivermectin treatment in mice. The idea is supported by other study which reflect the ability of the ivermectin at a dose 300 micrograms /Kg. B . Wt . to enhance antibody production and lymphocytes number resultant in treatment the early formed bovine warts not in advance stages (Jameel *et al.*,2011). One of the components of the activated lymphocytes is T – cell , it can be distinguished from others by presence of special receptor on it's surface called T cell receptor (TCR) and play a central role in cell – mediated immunity (Schwarz and Bhandoola ,2006) . The activated lymphocytes had potent effect in destruction of fungal cells and facilitate the lesions cure and skin improvement in less time and, agree with Gudding and Lund (1995) proved that fungal elements in infected areas did not decrease until both humoral and cellular responses had been established.

There is significant depression ($p<0.05$) in monocyte cells rate, so the mean values were depressed from the normal values 4.300 ± 1.400 % before treatment and reaches to 2.200 ± 1.152 % after treatment. These results didn't have any side effects on the animals health along the period of treatment and the values were returned to normal after clearance of the lesions as shown in the Table(2).

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دراسة فعالية الأيفرمكتين في علاج داء القرع Dermatophytosis في الأبقار.

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المستخلص

جمعت 20 عينة من قشطات من جلد الأبقار التي أظهرت وجود علامات لأمراض جلدية من بعض الحقول من مختلف المناطق في محافظة ديالى للفترة من تشرين الثاني 2012 ولغاية مايس 2013.

أظهرت نتائج العزل بأن 18 عينة من مجموع 20 وبنسبة 90% كانت موجبة للعزلات الفطرية. كانت أعلى نسبة للخمج في شهر تشرين الثاني وبنسبة 50% ، 30% في شهر شباط ، بينما لوحظت أقل *Trichopyton* نسبة في شهر آذار وبنسبة 20%. العزلة الرئيسية المعزولة . تم إعطاء الأيفرمكتين

يمثل الفطر *verrucosum* بجرعة 200 مايكروغرام \ كيلوغرام من وزن الجسم تحت الجلد لكل

الحيوانات المصابة. لوحظت علامات الشفاء بعد مرور 10 أيام من الحقن وذلك بسقوط القشور المتواجدة على الجلد وظهور الشعر. مما وجد عكس أهمية استخدام الأيفرمكتين بنجاح في علاج الداء في الأبقار نتيجة التنشيط المناعي ممثلاً بارتفاع العدد الكلي لخلايا الدم البيضاء (الخلايا اللمفية) بعد استخدام الأيفرمكتين.

الكلمات المفتاحية: الأبقار، التهاب الجلد الفطري، القوباء، الأيفرمكتين .