Hematological and Histopathological Effects of Ivermectin in Treatment of Ovine Dermatophytosis in Diyala Province-Iraq

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Abstract: This study conducted to determine the hematological and histopathological effect of ivermectin in treatment ovine dermatophytosis in Diyala province. Fifty scraping samples were collected from sheep (ewes, lambs) which showed clinical signs of skin diseases from some farms in different areas of Diyala province. Out of 50 skin scraping of sheep 40 (80.0%) were positive for ringworm. Two species of dermatophyte sp. were isolated Trichophyton verrucosum and Trichophyton mentagrophytes. The highest incidence of infection was recorded in March (100.0%), February (87.5%) compared with (66.66%) in November and December. Recovery was observed after 13 days of single subcutaneous injection of 200 mg/kg ivermectin. The hematological effect of ivermectin show elevation in the eosinophil, monocytes and neutrophils rate when compared with normal values. The histopathological effect of ivermectin show that the affected area of the skin after treatment reflects by presence of perivascular cuffing; appear of keratinization; presence of few amount of fibrous connective tissue; presence of large amount of macrophages & eosinophil and neutrophils. In conclusion, ivermectin can be used successfully and effectively in the treatment of ovine dermatophytosis, minimizing the duration and the cost of treatment in addition to other uses in treatment of mange, skin lice and some endoparasites.

Keywords: Hematology, Histopathology, Ovine dermatophytosis, Ivermectin

1. Introduction

Dermatophytosis (ringworm) in sheep is a zoonotic skin infection of keratinized tissues caused by several types of fungus. The fungus causing ringworm is likely most often transmitted when infected sheep are closely sheared releasing spores attached to wool shafts into the air. Nearby lambs with no wool, little lanolin, and irritated skin are very susceptible. Ringworm can also be spread by contact with animals, clipping, brush, cards, lambs tubes and blankets, fence posts, wire, and the hands of handlers[1]. For each animal species, the dermatophytes involved depend on the host studied and on the geographical and environmental conditions. Trichophyton verrucosum and Trichophyton mentagrophytes are the usual zoophilic dermatophyte involved in sheep ringworm throughout the temperate regions of the world[2]. The animal age and trauma are important predisposing factors of disease[3]. Sheep ringworm mainly occurs in lambs and is rapidly spread in the herd via infected propagates, example hyphae, and specialized fungal spores named arthrospores.[4]. The disease occurs worldwide and T. verrucosum is the almost exclusive etiologic agent [5]. Spores may survive in the environment for 2 to 3 years [6]. Aside from animal involvement, several human outbreaks of T. mentagrophytes infection have been reported in the face and the body, so far by direct contact with infected animals or indirect contact with infectious propagates in the environment [7, 8]. 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[9].

One to four weeks after coming into contact with the fungus, sheep will first show signs of disease. The fungus affects both the outer layers of skin and the wool. The fungus spreads outward from the center causing a more or less circular area of wool loss. The ears, head, loin and neck are the most likely areas to be affected[10]. The first sign of ringworm is often a raised area where the wool is clumped and feels stiff. These areas are more easily felt than seen. Once the wool comes out, a circular area of wool loss 2 to 2 inches in diameter is seen[6]. Often, this area is covered by a gray-white scab. Itching is not usually present. Almost all animals with ringworm will recover in 1-4 months, though a spot of darker wool may result. It is sometimes difficult to diagnose club lamb fungus in animals in fleece; therefore, shearing assists in accurate diagnosis [4].

Animals cases of ringworm infection have been successfully treated by different antifungal agents such as azole compounds, iodine preparation as local application, and griseofulvin orally[11]. Commercial vaccines containing an attenuated strain of T. verrucosum (LTF-130 or CCM 8165) have been used successfully for the control of bovine dermatophytosis in Europe and Russia [12]. Ivermectin is macrocyclic lactones are products or chemical derivatives of soil microorganisms belonging to the Streptomyces avermitilis fungus. The main uses of ivermectin in treatment of intestinal helminthes infections as strongylol-
biopsy is removed from affected area before treatment and under local anesthesia, a small plug of skin called a punch biopsy is taken from the peripheral or edge of the lesion, then collected into sterile Petri dish and transmitted to the laboratory under aseptic conditions. The specimens are treated with 10% KOH to dissolve tissue material, leaving the alkali-resistant fungi intact, or stained with special fungal stains [14, 15].

The medium was prepared and poured into sterile Petri dishes for isolation or kept in slant screw caped bottles (universals) for maintaining the isolates. Some of prepared Petri dishes had been taken and by sterilized disposable syringe; ten, twenty and forty ml of 0.2 mg/100 ml ivermectin solution were pulled and added to culture medium to obtain three concentrations 0.001%, 0.02% and 0.04% and serves in refrigerator till use.

Each sample was inoculated directly on two sabouraud dextrose agar (SDA) (PH 6.9) with the addition of 0.05g/liter chloramphenicol and 0.4g/liter cycloheximide which incubated in the incubator at 37°C 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. After colony identification, and to demonstrate the effect of ivermectin in vitro; small parts from one of the causative fungi colony (T. mentagrophytes) under aseptic condition were be taken and cultured in the center of the media mixed ivermectin and incubated in the incubator at 37°C for (1-4) weeks.

2.2 Skin Biopsy

Under local anesthesia, a small plug of skin called a punch biopsy is removed from affected area before treatment and after recovery then fixed in (10%) formalin for histopathology.

2.3 Identification of Fungi

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

2.3.1 Ivermectin

Is manufactured by Merco Vet. Company Belgium and used by two ways; the first is injected to the infected animals subcutaneously and second, added to the culture medium.

2.3.2 Animal groups

Infected animals were divided into two groups:

a) Group: (Ivermectin treatment) 25 sheep (at different age and sex) were treated at a dose 200 micrograms /Kg. B.W. by subcutaneous injection; the treatment depend upon the clinical signs. So the common dose was be 200 micrograms /Kg. B.W [16] (Bogan and Mckellar, 2001). The dose is repeated after 11 days according to the information which had been taken from the manufacture corporation if need.

b) Group: (Iodine treatment) 25 sheep (at different age and sex) were treated by 2% iodine ointment by local application once daily carefully conducted using gloves, because of a possible infestation of the worker [17].

2.3.3 Collection of blood sample

After disinfected area of jugular vein, five milliliters of blood was be collected before and after of ivermectin treatment from group (a) animals and they are reserves in EDTA tube to determinate the hematological changes of blood parameter.

2.4 Statistical Analysis

The differences are compared by using (F-Test) at p<0.05 and Chi-Square (version 12.) [18]

3. Results

Fifty skin scraping of sheep 40 (80.0%) were positive for ringworm. The highest percentage of infection was seen in March (100.0%), February (87.5%), while the lowest percentage seen in November and December (66.66%) as in table (1).

The present study show high percentage of infection recorded in young sheep (lambs) than adult sheep (ewes) with significant at (P<0.05) as in table (2). Recovery was observed after 13 days from single injection of ivermectin at a dose of 200 mg/kg. S/C. was administered to sheep started by scales dropping and appearance of the hairs with significantly important (p<0.05) when compared with iodine preparation administrated locally so the recovery was observed after 30 day, as in figure (1). The clearance of lesion period differed between two group of treatment with significant difference P<0.05 as in table (3). The hematological changes show decrease in Hb, PCV value and significant difference (P<0.05) in differential white blood cells as in table (4). Two species of dermatophyte sp. were isolated Trichophyton verrucosum and Trichophyton mentagrophytes, as in figure (2). The result of inhibitor test show significant difference between the concentrations of ivermectin on T. mentagrophytesa growth in vitro as in table (5) and figure (3).
Histopathological effect of ovine dermatophytosis show that the affected area of the skin before treatment by ivermectin which reflects three tissue changes, the first is hyper keratinization of the epidermis, the second is the presence of few inflammatory cells resemble by neutrophils and monocytes and the third is few fibrous connective tissue. Whereas, the affected area of the skin after treatment by ivermectin which reflects four tissue changes; the first is incomplete healing of the epidermis; the second is the presence of high number of inflammatory cells resemble by eosinophils, monocytes and neutrophils; the third is too much fibrous connective tissue and prevascular cuffing cells as in figure (4 a-b).

Table 1: Frequency distribution of ovine dermatophytosis according to months of study

<table>
<thead>
<tr>
<th>Months</th>
<th>No. of Examined samples</th>
<th>No.(%)of Positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>November 2013</td>
<td>6</td>
<td>4(66.66%)</td>
</tr>
<tr>
<td>December 2014</td>
<td>6</td>
<td>4 (66.66%)</td>
</tr>
<tr>
<td>January 2014</td>
<td>8</td>
<td>6 (75%)</td>
</tr>
<tr>
<td>February 2014</td>
<td>8</td>
<td>7(87.5%)</td>
</tr>
<tr>
<td>March 2014</td>
<td>8</td>
<td>8 (100%)</td>
</tr>
<tr>
<td>April 2014</td>
<td>8</td>
<td>6 (75%)</td>
</tr>
<tr>
<td>May 2014</td>
<td>6</td>
<td>5(83.33%)</td>
</tr>
</tbody>
</table>

Total | 50 | 40(80%) |

X²= 3.906 ; P< 0.05 , NSD

X²= Chi-Square, NSD = No Significant difference

Table 2: Frequency distribution of sheep with ovine dermatophytosis lesions according to age

<table>
<thead>
<tr>
<th>Age</th>
<th>No. of Examined samples</th>
<th>No.(%) of Positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-12 month</td>
<td>30</td>
<td>27 (90%)</td>
</tr>
<tr>
<td>2-5 years</td>
<td>20</td>
<td>13 (65%)</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>40 (80%)</td>
</tr>
</tbody>
</table>

X²= 4.688 , P< 0.05 , S.D.

X²= Chi-Square, SD = Significant difference

Table 3: Type of treatment and the time of clearance of ovine dermatophytosis lesions

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Treatment Dosage</th>
<th>Period of clearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ivermectin treatment</td>
<td>1 % Ivermectin</td>
<td>13-24 Days*</td>
</tr>
<tr>
<td>Iodine treatment</td>
<td>2% Iodine oint-</td>
<td>30-40 Days</td>
</tr>
</tbody>
</table>

Table 4: Heamatological change of sheep with ovine dermatophytosis treated by ivermectin

<table>
<thead>
<tr>
<th>The parameters</th>
<th>Normal range</th>
<th>Before treatment S.D</th>
<th>After 11 days of treatment S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Packed cell volume (PCV) (%)</td>
<td>24-45</td>
<td>23 ± 2.68</td>
<td>22 ± 1.26</td>
</tr>
<tr>
<td>Hemoglobin (Hb) (g/dL)</td>
<td>8-15</td>
<td>7.6 ± 0.087</td>
<td>7.4 ± 0.196</td>
</tr>
<tr>
<td>Platelets (Per/ µL)</td>
<td>250000 - 750000</td>
<td>795000 ± 1.34</td>
<td>865000± 1.46</td>
</tr>
<tr>
<td>Red blood cell (RBC) (x10⁹) µL</td>
<td>9.0-15.0</td>
<td>8,755,250± 1.22</td>
<td>7,883,350± 1.32</td>
</tr>
<tr>
<td>Mean corpuscular volume (MCV) (fl)</td>
<td>28-40</td>
<td>26± 1.41</td>
<td>27±0.632</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin (MCH) (Pg)</td>
<td>8.0-12.0</td>
<td>8 ± 1.264</td>
<td>9 ± 1.032</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin concentration (MCHC) (g/L)</td>
<td>31.0-34.0</td>
<td>33± 0.632</td>
<td>33.6 ± 0.752</td>
</tr>
<tr>
<td>White blood cell count (WBC) (Per/µL)</td>
<td>4000-12,000</td>
<td>5125± 0.418</td>
<td>5.138 ± 0.422</td>
</tr>
<tr>
<td>Neutrophils %</td>
<td>20-42</td>
<td>43± 0.894</td>
<td>45 ± 1.560</td>
</tr>
<tr>
<td>Lymphocytes%</td>
<td>40-75</td>
<td>39± 1.366</td>
<td>24 ± 0.830*</td>
</tr>
<tr>
<td>Monocytes %</td>
<td>0-6</td>
<td>8±1.264</td>
<td>10 ± 0.152*</td>
</tr>
<tr>
<td>Eosinophils %</td>
<td>0-10</td>
<td>9±0.632</td>
<td>20 ± 1.092*</td>
</tr>
<tr>
<td>Basophils %</td>
<td>0-3</td>
<td>1±0.582</td>
<td>1± 0.582</td>
</tr>
</tbody>
</table>

M= Mean , SD= Stander division , Significant difference (P<0.05)

Table 5: Evaluation of different concentrations of ivermectin in T. mentagrophytes as growth in vitro

<table>
<thead>
<tr>
<th>No.</th>
<th>Concentrations</th>
<th>1- test value (1,2)</th>
<th>1- test value (1,3)</th>
<th>1- test value (2,3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.001%</td>
<td>26.77*</td>
<td>87.42*</td>
<td>40.0*</td>
</tr>
<tr>
<td>2</td>
<td>0.02%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.04%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*significant difference at (P<0.05)

Figure 1: Reveals the efficacy of ivermectin in treatment of ovine dermatophytosis and show the clearance of the lesions in the lambs with hair regrown. A: before treatment, B: After treatment.
Figure 2: A: The shape of completely grow colony of *T. verrucosum* fungi on (SDA) at 37°C. B: The shape of completely grow colony of *T. mentagrophytes* fungi in (SDA) at 37°C.

Figure 3: Shows the inhibitory effect of three concentrations of ivermectin on *T. mentagrophytesas* growth in vitro. Plate No.1, the concentration mean = 0.001% Plate No.2, the concentration mean= 0.02% Plate No.4, the concentration mean= 0.04%.

Figure 4(a): Revealed the histopathological section of affected area of the skin before treatment by ivermectin. (20 X). Figure 4(b): Revealed the histopathological section of affected area of the skin after treatment by ivermectin.(40X)

4. Discussion

The high percentage of ovine dermatophytosis 80% may indicate endemic nature, environmental conditions, overcrowding and continuous exposure to the causative agent which agree with opinion of others [19, 20]. Higher percentage of infection in young sheep than adult sheep may attributed to animals age, sucking of lamb from infected ewes. This opinion comes in concordance with [3], they reported the age and trauma are important predisposing factors of disease and the infection mainly occurs in lambs and is rapidly spread in the herd via infected propagates.

After culture and examination, *T. verrucosum* represent the majority of isolates characterized by chlamydomspores in-chain; rare macroconidia. *T. mentagrophytes* represents the minor isolates that reveals the presence of chlamydomspores, microconidia and macroconidia. These results make us to say, the *T. verrucosum* is the usual cause of ringworm in sheep that agree with[21] who proved that *T. verrucosum* isolated from 70% of ram lambs. *T. mentagrophytes* and *T. verrucosum* equally cause ringworm infection of sheep [19].

In the present study we used the injectable ivermectin as biological source of treatment more benefit and less side
effect, and better than chemical drugs which are used locally. [22]elicits the side effects of some topical antifungal drugs as amphotericin B and ketoconazole and miconazole which cause anorexia, vomiting, seizures, and cardiac arrest, which have been reported with the use of amphotericin B. Ketoconazole may produce hepatotoxicity. Miconazole may produce tachycardia, arrhythmias, fever, nausea, and thrombophlebitis after intravenous administration. [13] were ensures that mammals can ingest ivermectin with a high degree of safety.

Significant difference (p<0.05) was recorded in duration of treatment (13-24 days) with ivermectin compared with topical antifungal such as iodine compound which needs more time for clearance between 30-40 days and needs high cost [17].

The hematological changes of ivermectin in blood parameter show significant elevation (P<0.05) in the rate of eosinophils and monocytes in addition to activation of the neutrophils after treatment by ivermectin which may attributed to the effect of ivermectin on stimulation of cellular immune response of the animal. This explanation come in concordance with[23]. They recorded these cytotoxic materials which released by stimulated eosinophils may be cause damage in the cell membrane of the fungus and facilitate the drug entrance inside the target cells with its cytotoxic effect[24], mentioned that eosinophil granules cationic proteins induce tissue damage and dysfunction. [25] had been proved the ability of dendritic cells to phagocytose or engulf the two proliferative forms of Aspergillus fumigatus the conidia and hyphae.

This study agree with[26] which 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 refers to the role of cellular branch of immune system in protective immunity against dermatophyte infections [27, 28]. The idea is supported by other study which reflect the ability of the ivermectin at a dose 300 mg/Kg. B.W to enhance antibody production and lymphocytes number results in treatment the early formed bovine warts not in advance stage[29]. This result didn’t has any side effects on the animals health along the period of treatment because the ivermectin is nationally used in treatment of the endo and ectoparasites in ruminants. This study was agree with[15] which proved the ability of ivermectin in treatment of ringworm infection in cattle. This study disagree with[30] mentioned that ivermectin have no antibacterial or antifungal activities although it is structurally similar to macrolide antibiotics and antifungal macrocyclic polyenes. This structural similarity of macrolides may leads to this effect on the causative agent. Our study was supported by in vitro experiment that revealed the effect of inoculated ivermectin to Trichophyton mentagrophytes fungus culture. These findings proved that ivermectin could be used successfully and effectively in the treatment of ovine dermatophytosis, minimizing the duration and the cost of treatment in addition to other uses in treatment of mange, skin lice and some endoparasites.

References


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